Objection to the application from Lilongwe University of Agriculture and Natural Resources' Bunda College for confined field trial of genetically modified pod borer-resistant cowpea varieties

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This objection, prepared by Commons for EcoJustice (EcoJustice), is supported by:

EcoJustice, Civil Society Agricultural Network, Farm Radio, Coordination Union for the Rehabilitation of the Environment, Self Help Africa, Churches in Action Relief and Development, Find Your Feet, Trustees of Agricultural Promotion Programme, Farmers Forum for Trade and Social Justice, Network for Youth and Development, Kusamala Institute for Agriculture and Ecology, Never Ending Food, Right to Food, Evangelical Lutheran Church in Malawi, Malawi Organic Growers Association, Sustainable Rural Growth and Development Initiative, Schools and Colleges Permaculture, Afriseed and Women in Agribusiness in the Sub-Sahara Africa Alliance and all members of the GMO-Free Malawi platform (a grouping of individuals, farmer groups, organisations, networks and faith-based institutions who share common environmental, safety and scientific concerns about GMO crops in general and unify their voice to advance a campaign against any intent by profit-oriented entities to introduce GMOs in Malawi).

EcoJustice, a not-for-profit non-governmental organisation established in Malawi, is an environmental-rights bureau that focuses on the core themes of biodiversity, seed sovereignty and community rights, and climate and food justice. It aims to strengthen environmental rights-based approaches and to interrogate related policy-development processes and associated legal instruments designed to affect biodiversity and farmer-managed seed and knowledge systems. EcoJustice works in partnership with like-minded non-governmental organisations, faith-based institutions, government institutions, community-based organisations and networks to advocate and lobby for the incorporation of policies, laws and regulations supportive of food and seed sovereignty in the sustainable-development mechanisms and decision-making processes taking place at the national and regional level. EcoJustice's secretariat office is in Lilongwe, Malawi.

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Acronyms and abbreviations

ACB:	African Centre for Biosafety
CFT:	Confined field trials
Ecojustice:	Commons for EcoJustice
GM:	Genetically modified
GMO:	Genetically modified organism
MLT:	Multi-locational trials

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1. Introduction

On 5 January 2015, Lilongwe University of Agriculture and Natural Resources' Bunda College placed a public notice in both the Nation and Daily Times newspapers announcing its intention to apply to the Malawi Biosafety Registrar for approval for a confined field trial (CFT) of genetically modified (GM) pod borer-resistant cowpea varieties. This notice is a significant event for Malawi, the region and the continent because cowpea is an indigenous African crop with great importance as a food security crop.

Cowpea (*Vigna unguiculata*) is a food and animal feed crop grown in the semi-arid tropical areas of Africa, Asia, Europe, the United States, Central America and South America. It originated and was domesticated in southern Africa, later spreading to East Africa, West Africa and Asia.[1] Cowpea is an important economic crop in many developing regions because of its high protein content, its adaptability to different types of soil and intercropping systems, its drought-resistance characteristics, and its ability to improve soil fertility and prevent erosion.[2] It is mostly small-scale farmers that grow it, often cultivating it with other crops. The sale of the stems and leaves as animal feed during the dry season also provides a vital income for farmers.

Cowpea is an African crop; nearly 96% of the more than 5.4 million tons of dried cowpeas produced worldwide originate from Africa. More than 11 million hectares are harvested worldwide; 97% of this in Africa.[3]

2. Summary of Civil Society's Objection

It is not clear from the CFT documents what the overall purpose of the CFT is. On the one hand it states the trial is to "evaluate the efficacy of the Bt resistance trait" while on the other claiming the goal "is ultimately to confirm substantial equivalence of Bt-cowpea". Unfortunately, the study's design will not confirm either: no experiment to compare Bt cowpea components with that of its conventional counterpart has been described, while the small-size and minimal replication of the CFT will not provide sufficient data on efficacy.

What little efficacy testing that is proposed is minimal and insufficient. The CFT design is lacking even the most basic parameters, proposing no methods to quantify Cry1Ab expression in the plant or its efficacy on the target pest. No consideration has been given to pest resistance evolution, or to effects on non-target organisms or secondary pests. The cowpea is known to attract beneficial insects, including wasps, honeybees and lady beetles.

It is our opinion that the measures proposed to prevent potential gene-flow between Bt cowpea and wild relatives in the surrounding area are inadequate. This is particularly alarming given the region's status as the centre of origin of cowpea.

The applicant claims that, based on field tests carried out previously in Nigeria, Ghana and Burkina Faso, 'no discernable phenotypic changes' were observed compared to non-transgenic cowpea plants. However, no data or reference to peer review study is made in support of this. Similarly, no data were provided to support claims that none of the genetic components were sourced from infectious agents and that none encode for allergens or toxins.

No data have been provided to support the applicant's claim that no changes in toxicity, allergenicity or plant composition are anticipated as a result of the genetic modification. Studies have previously shown that the Cry1Ab protein present in this Bt-cowpea variety can have toxic effects on non-target organisms, including mammalian species. Significantly, changes in plant composition, toxicity and allergenicity can arise from beyond the inserted sequence itself. Methods to detect such changes, including transcriptomics, proteomics or metabolomics analysis have not been used in this application. Furthermore, the applicant fails to provide basic information on the molecular characterization of the Bt cowpea event.

Evolution of resistance by target insect pests is the most serious threat to the continued efficacy of Bt crops. The development of insect resistance to Bt toxins in various parts of the world has been well documented, including field resistance of the stem borer (*Busseola fusca*) to Cry1Ab in South Africa. The CFT gives no consideration to non-target organisms, claiming Cry1Ab's mode of action is "highly specific for the larvae of lepidopteran insects such as *Maruca*". However, adverse effects of Bt toxins, in particular Cry1Ab, have been demonstrated on non-target organisms such as beneficial insects and water flea. Moreover, the cowpea is known to attract numerous beneficial insects. Finally, despite evidence from the USA and China that widespread Bt cotton adoption results in significant secondary pest pressure, there is no consideration of secondary pests in the application.

Cowpea is an important source of protein in for small-scale farmers in Malawi, as it can be grown in warmer, drier areas unsuitable for other leguminous crops and is typically the earliest available food during 'the hunger months'. Up to 90% of the cowpea grown in Malawi is from local, farm-saved varieties, with the vast majority of production being consumed on-farm. In Africa, farmer seed exchange has been shown to play a major role in gene flow in GM crops. Further, there is a thriving cross-border informal trade in seed and grains throughout southern Africa. The United Nation's World Food Programme (UN WFP) has previously recorded 'informal' cross border trade of cowpea between Mozambique and Malawi.

3. Background

3.1 Cowpea in Malawi

The cowpea is an important legume crop in Malawi and has been adapted to a wide variety of local conditions, particularly in the warmer, drier areas in the south of the country (the Lower Shire valley, Bwanje valley, Lakeshore and Phalombe plains, as well as dry plateau areas of Machinga).[4]

Table 1: Cowpea production in Malawi, 2000–2013

Year	Area harvested (hectares)	Yield (Kilogram/hectare)	Production (Million tons)
Average 2000-2013	89 886	515.5	50 249
2013	75 504	478.4	36 119
2012	70 599	435.4	30 736
2011	70 599	452.2	31 928
2010	67 196	392.2	26 352

Year	Area harvested (hectares)	Yield (Kilogram/hectare)	Production (Million tons)
2009	55 095	516.6	28 464
2008	81 187	645.9	52 437
2007	80 000	687.5	55 000
2006	119 048	496.2	59 067
2005	118 167	434.2	51 309
2004	105 984	550.1	58 306
2003	115 313	674.4	77 771
2002	108 351	630.9	68 354
2001	106 168	693.6	73 643
2000	85 188	633.9	54 000

Source: FAOSTAT.

Malawi's Agricultural Technology Clearing Committee approved three varieties of cowpea: "Sudan-1" and IT82Erl6, both released in 2003 by the National Cowpea Improvement Committee,[5] and ITK99-494-6, an *Alectra vogelii* resistant cowpea variety developed by the Department of Agricultural Research Services, Bunda College and the McKnight Foundation, in 2011.[6] In comparison, 14 varieties of groundnut, 30 varieties of the common bean, and six varieties of pigeon-pea have been released in Malawi.[7]

3.2 Cowpea agronomy

Cowpea is an early maturing crop, with days to maturity ranging from two to three months depending on variety and altitude, among other factors. In addition, it has a degree of tolerance to waterlogging[8] and is known as a nitrogen-fixing crop. Studies indicated that intercropping maize with cowpea leads to reductions in *Striga* infestations.[9] In a survey from Wageningen University, farmers said they selected cowpea because of its use as a food relish, it is easy to manage and it matures faster than many other crops.[10] In another research project centred on breeding *Alectra vogelii* resistant cowpea in Tanzania and Malawi, farmers were asked to select their most desired traits in cowpea. The most commonly sought after traits were large sized seeds (17%), resistance to *alectra* and producing high yields (both 15.1%), early maturing (13.2%) and taste preferences (11.3%).[11]

The principle parasitic weeds that attack cowpeas are *Striga gesnerioides* and *Alectra spp.*, while the major insect pests are pod-sucking bugs (*Riptortus spp., Nezara viridula* and *Acantomia sp.*), aphids (*Aphis fabae, Aphis craccivora*), blister beetle (*Mylabris spp.*) and pod borer (*Maruca vitrata*).[12]

In Malawi, both cotton and cowpea are grown in the same low-altitude and warmer areas and both are prone to insect pests. According to one report connected to the *Alectra vogelii* resistant cowpea project, "there is close complementarity in the growing of the two crops in that farmers may use sprayers earmarked for cotton for spraying in cowpeas."[13]

3.3 Processing, storing and consuming cowpea

Most cowpea is consumed where it is grown, with both the seeds and leaves used. The main form of consumption in Malawi and other parts of southern Africa is as boiled dry cowpea seeds (stew). The long cooking time (up to two hours, which presents time and energy challenges to consumers) and limited variety of cowpea-based products limits the wider use of dry whole cowpea seeds.[14] According to a survey, farmers rated the most important culinary traits associated with cowpea as faster cooking times, mashing and a cream colour.[15]

3.4 Background of the event

The application is for approval of a CFT of cowpea plants (*Vigna unguiculata* [L.] Walp) that have been genetically modified for resistance against an insect pest of cowpea in Africa, *Maruca vitrata*.

Cowpea line IT86D-1010 was genetically modified to express Cry1Ab protein encoded by the *cry1Ab* gene (Bt gene) sourced from *Bacillus thuringiensis*, a commonly occurring soil-borne bacterium.

The genetic elements in the event include:

1 The *cry1Ab* gene protein-coding region derived from *Bacillus thuringiensis* strain *kurstaki* HD1.
2. The *cry1Ab* gene promoter derived from the *Arabidopsis thaliana* gene for the small subunit of the enzyme ribulose bis-phosphate carboxylase.
3. The *cry1Ab* gene 3'polyadenylation signal derived from the *Nicotiana tabacum* gene for the small subunit of the enzyme ribulose bis-phosphate carboxylase.
4. The *nptII* (neomycin phosphotransferase II) gene protein coding region, derived from *Escherichia coli* (*E. coli*) transposon Tn5.
5. The *nptII* gene intron derived from the gene encoding the enzyme catalase from *Ricinus communis*.
6. The *nptII* gene promoter derived from the plant virus Subterranean Clover Stunt Virus gene 1.
7. The *nptII* gene 3'polyadenylation signal derived from the plant virus Subterranean Clover Stunt Virus gene 3.
8. *Agrobacterium* Binary Vector: DNA integration sequences derived from the T-DNA plasmid of *Agrobacterium tumefaciens*.

The proposed three-year trial will be conducted at Lilongwe University of Agriculture and Natural Resources' Bunda College campus in a specially designed CFT facility. Bt cotton field trials are being conducted as this same site. The trial size is 0.65 hectares of the two-hectare CFT site; the remaining area will be allocated to cotton (presumably Bt cotton) and other rotational crops such as maize.

4. The Confined Field Trial

4.1 What is the purpose of the CFT?

It is important that the applicant clearly describe at the outset the main objective of the CFT application. This will determine the correct experimental design and methodologies to apply in order to achieve the objective. However, the application does not make the objective clear. As indicated below, while the objectives are not conflicting, they do require completely different experimental designs.

On page 7: "This effort is currently at the advanced research and proof-of-concept phase and hence this new application seeks to test genetically modified cowpea plants to **evaluate their efficacy against *Maruca*** [emphasis added] under field conditions in Malawi." And on page 14:

“The objective of this CFT is to carry out confined field trials with Bt cowpea to **evaluate the efficacy of the Bt resistance trait** [emphasis added]against *Maruca vitrata* strains present in Malawi, and to determine the yield response of cowpea to protection from *Maruca* damage.”

However, the application states on page 12: “It is worth noting that our goal in conducting this CFT and subsequent MLT [multi-locational trials] in Malawi (and in other countries) is ultimately to **confirm substantial equivalence** [emphasis added] of Bt-cowpea, based on internationally-accepted principles and guidelines for food and feed safety assessment as stipulated by the Codex Alimentarius Commission (Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants [CAC/GL45-2003]) and other risk assessment procedures so that smallholder African farmers may benefit from this technology.”

According to the Codex Principles for the “Risk Analysis of Foods Derived from Modern Biotechnology”, the substantial equivalence concept is used to identify similarities and differences in composition between the new GM plant and its conventional counterpart and aims to identify potential safety and nutritional issues resulting from any identified differences. In addition, the safety assessment addresses a multidisciplinary approach that takes into account both intended and unintended changes that may occur in the plant or in the foods derived from it. It should be noted that substantial equivalence does not substitute for safety testing; it is merely the beginning of the safety assessment.

The applicant **did not describe any experiment aiming to compare and analyse Bt cowpea components with its conventional counterpart** in order to achieve the second objective related to substantial equivalence analysis. The experiments described in this CFT application are based on phenotypic characterisation of common agronomic attributes (i.e. plant emergence, plant height, flowering date, total weight of seeds per plant, pest incidence and so on). However, not all compositional differences between the GM plant and its conventional counterpart can be detected by the phenotype analysis. Genotypic changes in the GM plant may include changes in native/endogenous gene expression and regulation at the transcriptional, translational and post-translational levels. **Information on these aspects is regrettably lacking**.

The CFT design **does not allow efficacy to be studied reliably**, due to its small size and minimal replication, and the study of one transgenic versus three non-transgenic varieties would introduce unrelated variability and noise into the system that could mask any potential significant differences between the GM and isoline non-GM cowpea.

Furthermore, **the quality of the efficacy testing proposed is minimal and insufficient** to understand how the expressed levels of Cry1Ab toxin affect the target pest. Efficacy testing should also be from the perspective of the pest damage to the plant, where the endpoints tested are plant parameters such as feeding damage on the plant.

The CFT application does not consider the following at all:

- **There are no data requirements for the efficacy of the Bt toxin on the target pest and no proposed quantification of the Cry1Ab toxin in plants to see if the toxin production coincides in effective levels with the most damaging life stages of the pest or it fulfils even the minimum requirements for pest resistance evolution management, such as high dose.**
- **There are no endpoints to be measured of the target pest, there is no consideration of efficacy on the target pest or its potential to evolve resistance.**
- **There is no consideration of the aspect of pest resistance evolution, perhaps the most important question when it comes to Bt crops and their efficacy.**
- **There is no consideration of any environmental safety relevant aspect, such as effects on non-target organisms, effects on secondary pests, and biodiversity or food chain effects.**

4.2. Gene flow and containment measures

Confinement or containment measures are used to prevent escape of the GM plant to the broader environment and to reduce exposure to humans and animals. In order to reduce exposure to a genetically modified organism (GMO), two critical knowledge determinants need to be understood and tested: (i) determine exposure routes and (ii) demonstrate ability to detect and monitor the GM plant at levels that would prevent it from causing harm.

To begin, the applicant should indicate what containment measures are to be used. Then, the applicant should indicate how those measures would be assessed as appropriate and, if the CFT is approved, how the applicant will measure their effectiveness. The risk assessment should then include an assessment of the risks of escape with measures in place to evaluate whether the containment measures will make the risk manageable.

Regarding genetic confinement, the applicant claims that “the absence of compatible species around the CFT site as well as the very low out-crossing rate of cowpea provide a high degree of assurance that pollen-mediated gene flow will not occur” (p.19). The applicant provides three additional measures to this CFT that are related to (i) a perimeter border of three rows of conventional cowpea, (ii) in the first year of the CFT, the trial plot will be netted and (iii) spatial isolation distance of 30 metres will be maintained beyond the CFT plot, which will be monitored and maintained free of compatible cowpea plants of any kind.

These measures take into account potential gene flow between Bt cowpea and wild spontaneous cowpea in the surroundings of the CFT plots, as well as pollinators visiting the plot. For the purposes of a case-by-case risk assessment, however, **additional factors should be considered**: Malawi territory is considered part of the centre of origin of cowpea[16] and out-crossing levels, even when considered negligible, should be avoided. For instance, the literature cited in the application that supports that negligible levels of out-crossing were observed could be contested by another more robust study that has examined out-crossing rates and genetic structures in 35 wild cowpea populations from West Africa, using 21 isozyme loci[17]. This study reports out-crossing rates that range from 1% to 9.5% and the analyses of both the genetic structure of populations and the relationships between the wild and domesticated groups suggest possibilities of gene flow that are corroborated by field observations.

Therefore, the isolation of Bt cowpea fields from visiting pollinators should be performed during all years of the proposed CFT. Larger distances (> 30 metres) should be monitored on a frequent basis in order to prevent seed or pollen dispersal by animals.

Some of the information provided by the applicant could be used to develop tools for detection and monitoring. For example, developing transgene detection and identification methodologies needs the description of insertions in order to monitor GMO spread into the environment. In addition, description of insertions and transgene expression is relevant to characterising transgene persistence in the environment.

However, the application does not show the detection sensitivity and the ability of the monitoring programme to operate in time scales necessary to mitigate risk.

5. Information about the Modified Plant

5.1 Phenotypic changes

The applicant claims that the intended phenotype is resistant to feeding damage caused by *Maruca* pod borer. It also states that "many years of field trial testing conducted with Bt-cowpea in different genetic backgrounds, and across a range of environments in Nigeria, Ghana and Burkina Faso, indicate that there are no discernible phenotypic changes in the modified plant compared to non-transgenic cowpea plants" (p.11). However, the application provides no data or reference to a previous peer-reviewed study and thus **the claim cannot be verified**.

5.2 Source of the genetic material

The applicant claims that none of the genetic components are infectious agents, and none of them encode allergens or toxins (p.12). The applicant provides a list of all genetic elements (i.e. transgene coding sequence, promoter, terminator, intron, and so on). However, **no data were provided** for the claim that the genetic components were not sourced from infectious agents, and that none encode allergens or toxins.

In fact, the cry1Ab toxin has already been described as having potential allergenic and toxic effect to mammals. A recent study showed BALB/c mice-specific antibody response after intranasal installation exposure to purified Cry1Ab protoxin and toxin preparations. This **new potential exposure route** demonstrates the principle that these proteins may affect the immune system; in other words, they may act as immunogens, after intranasal administration. In addition, the elicitation of specific IgE antibodies indicates allergenicity of the purified Cry1Ab proteins.[18] In addition, the toxic effect of Cry proteins to non-target organisms is well known (see section 6.2).

5.3 Changes in toxicity, allergenicity or plant composition

The applicant claims, "No changes in toxicity, allergenicity or plant composition are anticipated by the genetic modification. Neither Cry1Ab nor nptII possess similarities to allergens, and neither exhibits mammalian toxicity. Both the genes and the protein products expressed in Bt-cowpea have previously undergone rigorous characterization in genetically modified crops that are in commercial release" (p.12). However, **no data were provided for this claim**.

For a case-by-case risk assessment framework, the allergenicity of Cry1Ab and NptII cannot be assumed through comparison to expression in other kinds of plants. Post-translational modifications, particularly glycosylation, can be species- and environment-specific.

In fact, Cry1Ab protein has been shown to have toxic effects to non-target organisms (see section 6.2). In addition, studies have shown that modified Bt toxins are not inert on non-target human cells, and that they can present combined side effects with other residues of pesticides specific to GM plants.[19] For example, studies conducted on rats fed Bt corn varieties containing Cry1Ab have shown signs of toxic effects on liver and kidneys.[20] Furthermore, old and young mice fed Bt corn MON810 (containing Cry1Ab) showed marked disturbance in immune system cells and in biochemical activity.[21]

Most importantly, changes in plant composition, toxicity and allergenicity might not only arise from transgene products (transgenic protein and transgenic RNA species).[22] Other toxic and allergenic effects might also arise from plant components that are not expressed from inserted sequences, but might have been altered by them.

The molecular characterisation of Bt-cowpea did not include, for instance, a targeted search for RNA produced from the 3' and 5' ends of the insert. The description of the transcripts can help to resolve uncertainties about the potential to make unintended new proteins or for the arising of unintended regulatory RNAs and then to determine if either kind of gene product might cause adverse effects.

RNA has a very powerful role in gene regulation separate from its role as an intermediate in protein production. This role is still not understood fully nor is the biochemistry of RNA-guided processes completely described. There may be times therefore when uncertainties in the hazard identification step can only be reduced using transcriptomics.[23] A non-targeted transcriptomics approach can identify both unintended RNA species that arise indirectly from the genetic engineering and can help to detect quantitative changes in some RNA species, thus helping to narrow down the search for other unintended changes in the GM plant.

To our knowledge, a full transcriptomic analysis has not been done for pre-market approval of any commercial GM plant to date.[24] Nevertheless, it remains the prerogative of regulators to ask for such analyses if their legislation is consistent with the Codex Alimentarius Guidance.[25,26]

In addition, it has long been known that proteins are a class of molecule that has the potential to cause adverse effects. While most proteins are benign and even beneficial, some can be toxins or cause immune responses in specific species or individuals, including powerful allergic responses. Therefore, there may be times when uncertainties in the hazard identification step can only be reduced using non-targeted proteomics.[27] A non-targeted proteomics approach can identify both unintended protein species that arise indirectly from the genetic engineering and can help to detect quantitative changes in some protein species, thus helping to narrow down the search for other unintended changes in the GM plant.

To our knowledge, a full proteomic analysis has never been done for pre-market approval any commercial genetically modified plant to date. Post-market analysis of selected crops and varieties has revealed some unanticipated changes.[28,29,30] Nevertheless, it remains the prerogative of regulators to ask for such analyses if their legislation is consistent with the Codex Alimentarius Guidance.

Other molecules than DNA, RNA and protein that are taken up, broken down or created in living things are designated metabolic compounds. There may be times when uncertainties in the hazard identification step can only be reduced using non-targeted metabolomics.[31] To our knowledge, a full metabolomic analysis has never been done for pre-market approval of any commercial GM plant to date.

However, manufacturers routinely measure selected compounds and report these as a "compositional analysis". This has always been done and is not designed particularly for a GM food-safety analysis. Crop breeders intend to make their product attractive to farmers and thus measure key constituents or anti-nutrients as part of their marketing strategy. Nevertheless, the compositional analysis, which is a limited form of a metabolomic analysis, has become a standard part of the regulatory dossier.[32]

Considering the variation of individual components between different varieties grown at different times and in different environments is valid provided that there is a single common variety grown at all locations and all times included in the comparison.[33] This is the role of the proper conventional comparator, which provides a baseline measure of what is physiologically possible for a variety with a history of safe use. There could be no examples of conventional organisms with all or a significant number of components simultaneously at the extremes of the combined historical and literature ranges. Those extremes combined into a single physiological package do not occur in crops with a history of safe use. "The appropriate comparators have all traits in common except for the newly introduced ones."[34]

The applicant does not mention any experimental analysis for the investigation of substantial equivalence of Bt-cowpea as described as the objective of this field trial.

5.4 Features of the genetic construct

The applicant only provided a genetic map for the vector used in *Agrobacterium*-mediated transformation. Therefore, it is not possible to know what sequences and their integrity have actually been inserted. **No description of the location of insertion and number of copies has been provided. The applicant therefore fails to provide basic information on the molecular characterisation of this event**. The lack of knowledge about basic genetic insertions prevents, for instance, the detection of transgene spread into environment.

6. Environmental Risk Assessment

6.1 Development of insect resistance

Evolution of resistance by the target insect pests is the most serious threat to the continued efficacy of Bt crops and field-evolved resistance has happened quickly in various parts of the world. This is true for some populations of five of 13 major pest species examined, compared with resistant populations of only one pest species in 2005.[35]

The introduced transgene *Cry1Ab* was the first Bt transgene used commercially on a large scale. It has already become ineffective against a whole range of target pest species all over the world, including in neighbouring countries such as South Africa. The use of *Cry1Ab* in the Bt cowpea is thus questionable, given that it may quickly become ineffective against the target insect pest, *Maruca vitrata*.

Insect resistance to Bt toxins has been documented in various parts of the world. For example, the field resistance by stem borer (*Busseola fusca*) in Bt maize (containing Cry1Ab) was first reported in 2007 in South Africa.[36] The resistance problems with Cry1Ab Bt maize have become widespread in the country, with resistant populations reported at new localities on a regular basis.[37] The South African experience shows that the predicted rate of resistance evolution in many instances is often underestimated.

Resistance development to other Bt toxins has been documented in different parts of the world. Resistant corn rootworm populations in Bt maize (containing Cry3Bb1) in the United States were reported in 2011, and as of 2014, resistance has been reported in four states.[38] In the United States, the frequency of resistance alleles has increased substantially in some field populations of cotton bollworm, *Helicoverpa zea*.[39] Monsanto confirmed pink bollworm resistance to Cry1Ac expressed in Bt cotton, in four districts in Gujarat, India in 2010.[40] A 2010 survey by Zhang et al. (2011) showed field-evolved resistance to Cry1Ac of the major target pest, cotton bollworm (*Helicoverpa armigera*), in northern China.[41] *H. armigera* has low susceptibility to Cry1Ab, which could hasten resistance development in this species.

6.2 Impact on non-target organisms

The CFT is conceived of strictly and solely as efficacy testing of the Bt cowpea, with no consideration given at all to target or non-target pests or non-target organisms in general. There is **no biosafety testing envisaged in regards to non-target pests**, as the application does not propose a relevant endpoint to be tested with non-target pests, including Lepidopteran non-target pests or other pest insects that are candidates for replacing the target pest *Maruca vitrata*.

The applicant ignores non-target organisms by claiming that Cry1Ab has a "well-understood mode of action [that is] is highly specific for larvae of lepidopteran insects such as *Maruca*" (p.11). However, scientific research is uncovering that the mode of action is less understood today than it was 20 years ago, and, today, at least three different models for Bt mode-of-actions are proposed.[42] The specificity of the mode of action is increasingly challenged, as more research emerges clearly documenting that far more species are affected by Bt toxins than previously believed. While originally Bt toxin efficacy was believed to be restricted only to some insect orders, Lepidoptera and Diptera mainly, this has successively been expanded to include up to six arthropod orders for which cross-active Bt toxins have been reported[43]. Notably, Cry1Ab toxin is the Bt toxin that bioassay data reports has affected species from six insect orders.

As expression of the Bt toxin in Bt plants is under the control of constitutive promoters, the Bt toxin is present in all plants parts throughout the entire plant's lifespan. Hence, all organisms (primarily arthropods, but also mammals and microorganisms) feeding on these plants will be exposed and, therefore, have a high likelihood of being affected by the toxin. Of particular concern is that cowpea is known to attract beneficial insects, including many types of wasps, honeybees, lady beetles, ants and soft-winged flower beetles.[44]

Adverse effects of Bt toxins, in particular Cry1Ab, have been demonstrated on non-target organisms such as beneficial insects (lady beetle[45] and lacewing larvae[46]), the aquatic water flea *Daphnia magna*,[47] which is an indicator species, and the earthworm, which is an important soil organism.[48]

Recent research has also documented the input of transgene products or transgene DNA into aquatic systems, headwater streams and rivers and connected them to possible adverse effects on some aquatic organisms. This is the case for Bt corn containing Cry1Ab, where pollen and detritus were shown to enter headwater streams and were subject to storage, consumption, and transport to downstream water bodies. Laboratory feeding trials further showed that consumption of the Bt corn by-products reduced growth and increased mortality of non-target stream insects, with possible unexpected ecosystem-scale consequences.[49]

6.3 Secondary pests

A secondary pest is one that under normal conditions does not present a serious problem, but becomes a serious problem following changes in management practices or disruption of control by a natural enemy. The application does not consider the issue of secondary pests occurring with reduction of target pest populations. This is a serious omission, as evidence from around the world shows that secondary pests may replace the target pest, necessitating increased pesticides sprays. For example, by 2002 mirids had became key insect pests in transgenic cotton fields in China and reports warned that the damage to cotton plants was likely to increase with further plantings of transgenic cotton, unless additional control measures were adopted.[50] By 2010, mirid bug population levels had progressively increased and acquired pest status in cotton and multiple other crops, in association with the regional increase in Bt cotton adoption.[51] A three-year study published in 2005 identified the emergence of another secondary pest, leafhopper, as its populations on Bt cotton were consistently larger than those on non-transgenic cotton.[52]

There are also reports of secondary pest emergence in the United States for Bt cotton. For example, *Farm Press* in February 2006 claimed that lygus was "slowly moving to the front of cotton industry's pest problems", and in March 2008, that cotton insect pressure had shifted. According to *Greenwire* (17 May 2010), "modified cotton curbs one pest only to unleash another".[53]

The applicant, in claiming that Bt cowpea will reduce pesticide use, states that "Reduced spraying for *Maruca* is also likely to allow populations of natural enemies to reach levels at which they contribute significantly to the control of important sucking pests such as aphids, thrips, red spider mites and white-fly. Outbreaks of these cowpea pests are often triggered by repeated spraying of broad-spectrum insecticides, forcing farmers into a vicious cycle of weekly spraying with very toxic chemicals." (p. 14). This is an implicit acknowledgement that such pests are already a significant problem in Malawi. However, there is **no consideration of whether secondary pest outbreaks will happen with the blanket expression of the Bt pesticide in cowpea**.

In addition, the belief that producing Bt crops will result in reduced pesticides applications has not borne out in actual Bt maize and Bt cotton production. Perhaps it reduced pesticides sprays for a transient period, but these increased again with the development of secondary pests. As there is a diverse cowpea pest complex, this means that single control strategies, such as Bt cowpea, are unlikely to produce satisfactory control measures.

The applicant should therefore be asked to provide a report identifying the potential secondary pests of cowpea in Malawi, an assessment of the potential for pest replacement to occur in the event that the Bt cowpea is planted, and the necessary risk management steps to address this risk.

7. Socioeconomic Concerns

While a consideration of socioeconomic concerns may seem premature at this stage of an application for CFT, it is important that these issues be considered at an early stage, given the importance of cowpea to Malawi as a food security crop, particularly for smallholders. If issues around important socioeconomic concerns are not anticipated, they may result in the suspension of approval at a later stage (for example, at commercialisation). If, at this stage, key questions around socioeconomic concerns cannot be answered or the threat to socioeconomic wellbeing becomes obvious, then increased investment and research efforts in Bt cowpea are not warranted.

Firstly, the value of Bt cowpea for Malawi should be evaluated. The target pest, *Maruca* vitrata is actually not a significant problem for farmers in Malawi. Biological control methods show enormous potential to control *Maruca* vitrata[54] and are more appropriate and sustainable alternatives.

7.1 Importance as a food security crop

On the national level, groundnuts, beans and pigeon-pea are planted on larger areas; however, cowpea is an important protein source in areas unsuitable for bean and groundnut production.[55] It has also been proposed as an important potential weaning food for infants and young children.[56]

Table 2: Area and production of legumes in Malawi

Legume	Area Harvested (Hectare)	Production (Million tons)
Groundnut (2002-2011)	254 478	227 089
Beans (2002-2011)	237 454	111 889
Pigeon-pea (2000-2013)	165 515	153 335
Cowpea (2000-2013)	89 886	50 249

Source: Groundnut & Beans, ICRISAT[57] (2013); Cow-pea & pigeon-pea, FAOSTAT.

In Malawi, women in particular value cowpea[58] because its green pods and leaves are the earliest food available during "the hunger months" prior to the main grain harvest. It also presents an opportunity for cash sales during this period (though other grains and legumes are traded in higher volumes). For the same reasons, cowpea is deemed a "crop of the poor".[59]

According to a 2010 AGRA farmer survey, 9% were growing cowpea, ranging from 3% in Dowa to 18% in Zomba. Slightly more women (10%) grew cowpea than men (8%).[60] Cowpea is a major Dimba crop grown in the Northern Lower Shire Valley. In this area, the poor do not typically have access to Dimba land.[61]

7.2 Cowpea seed systems and research

Most cowpea in Malawi is grown from local farm-saved seed. The Consultative Group for International Agricultural Research estimated that cowpea grown from improved seed accounts for only 10% of Malawi's 2009 cowpea harvest.[62] Results from more recent fieldwork from the African Centre for Biosafety (ACB) appear to support this because 87% of the farmers interviewed that grew cowpea (admittedly a small number) were using non-certified seed (74% of cowpea seed was replanted from the previous harvest).[63] In 2009/10, 2010/11 and 2011/12, one seed supplier provided cowpea seed for the subsidy programme, though little further detailed information is given.[64]

The Wageningen University-led Integrated Seed Sector Development programme classifies cowpea as being predominantly part of the non-governmental organisation/farmer organisation-led system, which encompasses formally certified, quality declared seed and informal/uncertified seed. This system is characterised by local exchange and distribution mechanisms, rather than any formal marketing channels,[65] though the Association of Seed Multiplication Action Group is also involved in seed multiplication and distribution of cowpea.[66]

In the formal system, foundation seed comes from the public-sector Department of Agricultural Research Services. There are about 5 metric tons of basic cowpea seed available in Malawi (compared to over 60 metric tons of bean seed and almost 210 metric tons of groundnuts), according to the Seed Trade Association of Malawi.[67]

7.3 Cowpea marketing issues

There is increased demand for cowpea for human consumption and for generating income; however, evidence suggests that production trends are declining. This is attributed to the high marketing costs associated with dispersed production and farmers' and traders' lack of price information along the market value chain.[68] Recent fieldwork conducted by ACB found that farmers cultivating cowpea were selling, on average, less than 25% of their total harvest.[69] In Malawi, about 80% of consumers and 90% of traders prefer brown seeds, while in Tanzania seed colour is not a determinant as most cowpea entering commercial markets is de-hulled prior to sale).[70]

Although most cowpea cultivated in Malawi is for home consumption, there have been reports of "informal" cross-border trade in cowpea occurring between Malawi and Mozambique, which grew 300 000 hectares in 2013. The United Nation's World Food Programme has estimated that an average of 10-12 metric tons a month was "informally" imported into Mozambique from Malawi between April 2006 and December 2007.[71]

More up-to-date cowpea-specific information is not available, but the same source states that from July to September 2012 over 20 000 metric tons of maize, rice and beans were informally traded across borders within the Southern African Development Community.[72]

7.4 Key questions

Some key questions that therefore could be asked are:

- How will Bt cowpea be priced/marketed in Malawi given that it is mostly grown from local/farm-saved varieties and given the lack of availability of officially certified varieties?

- What effect will replanting Bt cowpea have on the efficacy of the Bt protein in the following season (as cowpea is self-pollinating there will be little incentive for farmers to purchase fresh seed every year)?

- What are the implications of seed exchange for transgene spread or gene flow? The applicant states that cowpea has low out-crossing rates, and therefore there is little concern with transgene spread. However, it has been shown that farmer seed exchange in Africa plays a major role in gene flow of GM crops.[73]

- What are the gender implications of introducing Bt cowpea? Will farmers require credit to purchase Bt cowpea seed? In Malawi, cowpea is known as a "women's crop".

- Organic farmers use cowpea as a leguminous cover crop. Organic certification does not allow for the use of GM seeds. What would contamination of conventional cowpea with transgenes mean for organic farmers?

- Will the major buyers of cowpea (such as ADMARC or private companies) be happy purchasing Bt cowpea, given there are currently no GM food crops grown in Malawi?

- As stated in section 3.2, farmers grow cotton and cowpea in the same areas and are subject to similar insect pest pressure. If Bt cotton and Bt cowpea are grown in the same areas, what are the implications for pests to develop resistance?

- What are the implications for informal cross border trade of Bt cowpea seed into Mozambique and Zambia and the resulting biosafety implications of transgene spread?

8. Conclusion

Lilongwe University of Agriculture and Natural Resources (LUANAR) Bunda College has applied to the office of the Biosafety Registrar in Malawi to conduct a confined field trial (CFT) for a genetically modified (GM) cowpea variety, resistant to the insect pod borer (*Maruca vitrata*).

The cowpea is an important source of protein in Malawi as it can be grown in regions that are not suitable for the cultivation of other leguminous crops, such as beans and groundnuts. It is also the first crop to be harvested during the 'hunger months' when farmers are waiting for the main grain harvest.

The application itself is fatally flawed by poor study design and lacking even the most rudimentary biosafety data, such as basic information on the molecular characterisation of the event itself. Further assertions are made regarding phenotype changes, toxicity and allergenicity that are no supported with any data. Finally, despite a growing body of global evidence around insect resistance to Bt crops, adverse impacts on non-target organisms (including mammals) and increases in secondary pests associated with widespread Bt crop adoption, no consideration for these issues has been given in the application.

For these reasons, and other potential socio-economic concerns cited, the office of the Biosafety Registrar in Malawi should reject this application out of hand. In Malawi cowpea is a 'crop of the poor' and is overwhelmingly grown from local, farm saved seed. We urge our public agricultural research services to reflect this in their work, and strive to strengthen and improve varieties we have through participatory breeding processes with small-scale farmers that do not rely upon imported, expensive and highly risky technologies.

9. References

1 International Institute of Tropical Agriculture (IITA), http://www.iita.org/cowpea

2 IITA, ibid.

3 IITA, ibid.

4 Nkongolo, K.K., Bokosi, J., Malusi, M., Vokhiwa, Z. & Mphepho, M. 2009. Agronomic, culinary, and genetic characterization of selected cowpea elite lines using farmers' and breeder's knowledge: A case study from Malawi. *African Journal of Plant Science* 3 (7): 147-156.

5 Saka, A.R., Mtukoso, A.P., Daudi, A.T., Banda, M.H.P & Phiri, I.M.T. 2006. *Agricultural Technologies released by the Ministry of Agriculture and Food Security 2000-2005*. Lilongwe: Department of Agricultural Research Services, MAFS.
http://community.eldis.org/.59ee3fb9/Agriculture%20technology.pdf. Accessed 19/02/2015.

6 Hella, J.P., Chilongo, T., Mbwag, A.M., Bokosi, J., Kabambe, V., Riches, C. & Massawe, C.L. 2013. Participatory market-led cowpea breeding in sub-Saharan Africa: Evidence pathway from Malawi and Tanzania. *Merit Research Journal of Agricultural Science and Soil Sciences* 1(2):011-018.

7 ICRISAT. 2013. *Bulletin of tropical legumes*. http://www.icrisat.org/tropicallegumesII/pdfs/November-2013.pdf. Accessed 16/02/2015.

8 Directorate Agricultural Information Service. 2011. *Production guidelines for cowpea*. Pretoria: Department of Agriculture, Forestry and Fisheries.

9 Atera, E.A., Kondo, F. & Itoh, K. 2013. Evaluation of Intercropping and Permaculture Farming System for Control of *Striga asiatica* in Maize, Central Malawi. *Trop. Agr. Develop.* 57(4):114 –119.

10 Kamanga, B.C.G., Whitbread, A., Wall, P., Waddington, S.R., Almekinders, C. & Giller, K.E. 2010. Farmer evaluation of phosphorus fertilizer application to annual legumes in Chisepo, Central Malawi. *African Journal of Agricultural Research* 5(8): 668-680.

11 Kabambe, V.H., Mazuma, E.D.L., Bokosi, J. & Kazira, E. 2014. Release of cowpea line IT99K-494-6 for yield and resistance to the parasitic weed, *Alectra vogelii* Benth. in Malawi. *African Journal of Plant Science* 8(4):196-203.

12 Directorate Agricultural Information Service. 2011. *Production guidelines for cowpea*. Pretoria: Department of Agriculture, Forestry and Fisheries.

13 McKnight Foundation (2012) *Collaborative Crops Research Project No: 09-1206. Annex 2: Malawi country annual report 2012*. http://www.ccrp.org/sites/default/files/09-1206_year_2_2012_development_and_promotion_of_alectra_resistant_cowpea_cultivars_for_smallholder_farmers_in_malawi_and_tanzania.pdf. Accessed 16/02/2015.

14 http://twas.assaf.org.za/jspui/bitstream/123456789/27/2/01chapters1-2.pdf. Accessed 16/02/2015.

15 Nkongolo, K.K., Bokosi, J., Malusi, M., Vokhiwa, Z. & Mphepho, M. 2009. Agronomic, culinary, and genetic characterization of selected cowpea elite lines using farmers' and breeder's knowledge: A case study from Malawi. *African Journal of Plant Science* 3(7):147–156.

16 Ba, S., Pasquet, R.S. & Gepts, P. 2009. Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by. RAPD markers. *Genetic Resources and Crop Evolution* 51:539–550.

17 Kouam, E.B., et al. 2012. Genetic structure and mating system of wild cowpea populations in West Africa. *BMC Plant Biology* 12:113.

18 Andreassen, M., Rocca, E., Bøhn, T., Wikmark, O., van den Berg, J., Løvik, M., Traavik, T. & Nygaard, U.C. 2014. Humoral and cellular immune responses in mice after airway administration of Bacillus thuringiensis Cry1Ab and MON810 cry1Ab-transgenic maize. *Food and Agricultural Immunology* (in press) DOI:10.1080/09540105.2014.988128.

19 Mesnage, R., Clair, E., Gress, S., Then, C., Székács, A. & Séralini, G.-E. 2013. Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. J. *Appl. Toxicol*., 33: 695–699.

20 de Vendômois, J., Roullier, F., Cellier, D. & Séralini, G-E. 2009. A comparison of the effects of three GM corn varieties on mammalian health. *International Journal of Biological Sciences* 5(7): 706–726.

21 Finamore, A., Roselli, M., Britti, S., Monastra, G., Ambra, R., Turrini, A. & Mengheri, E. (2008). Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. *J Agric Food Chem*. 56(23): 11533–9.

22 Heinemann, J.A., Agapito-Tenfen, S.Z. & Carman, J.A. 2013. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. *Environ Int* 55:43–55.

23 Heinemann, J.A., Kurenbach, B. & Quist, D. 2011. Molecular profiling as tool for addressing emerging gaps in the comparative risk assessment of GMOs. *Environ Int* 37:1285–93.

24 Heinemann, J.A., Kurenbach, B. & Quist, D. 2011. Molecular profiling as tool for addressing emerging gaps in the comparative risk assessment of GMOs. *Environ Int* 37:1285–93.

25 CAC 2003a. *Guideline For The Conduct Of Food Safety Assessment Of Foods Derived From Recombinant-DNA Plants*. Codex Alimentarius Commission.

26 CAC 2003b. *Principles for the risk analysis of foods derived from modern biotechnology*. Codex Alimentarius Commission.
27 Heinemann, J.A., Kurenbach, B. & Quist, D. 2011. Molecular profiling as tool for addressing emerging gaps in the comparative risk assessment of GMOs. *Environ Int* 37:1285–93.
28 Zolla, L., Rinalducci, S., Antonioli, P. & Righetti, P.G. 2008. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. *J Proteome Res* 7:1850-61.
29 Agapito-Tenfen, S.Z., Guerra, M.P., Wikmark, O.G. & Nodari, R.O. 2013. Comparative proteomic analysis of genetically modified maize grown under different agroecosystems conditions in Brazil. *Proteome Sci* 11:46.
30 Agapito-Tenfen, S.Z., Vilperte, V., Benevenuto, R.F., Rover, C.M., Traavik, T.I. & Nodari, R.O. 2014. Effect of stacking insecticidal cry and herbicide tolerance epsps transgenes on transgenic maize proteome. *BMC Plant Biol* 14:346.
31 Heinemann, J.A., Kurenbach, B. & Quist, D. 2011. Molecular profiling as tool for addressing emerging gaps in the comparative risk assessment of GMOs. *Environ Int* 37:1285–93.
32 Heinemann, J.A., Kurenbach, B. & Quist, D. 2011. Molecular profiling as tool for addressing emerging gaps in the comparative risk assessment of GMOs. *Environ Int* 37:1285–93.
33 EFSA 2008. Safety and nutritional assessment of GM plants and derived food and feed: The role of animal feeding trials. *Food Chem Toxicol* 46:S2-S70.
34 Agapito-Tenfen, S.Z., Guerra, M.P., Wikmark, O.G. & Nodari, R.O. 2013. Comparative proteomic analysis of genetically modified maize grown under different agroecosystems conditions in Brazil. *Proteome Sci* 11:46.
35 Tabashnik, B.E., Brévault, T. & Carrière, Y. 2014. Insect resistance to Bt crops: lessons from the first billion acres. *Nature Biotechnology* 31:510–521.
36 Van Rensburg, J.B.J. 2007. First report of field resistance by stemborer, Busseola fusca (Fuller) to Bt-transgenic maize. *S. African J. Plant Soil* 24:147-151.
37 Van den Berg, J., Hilbeck, A. & Bøhn, T. 201). Pest resistance to Cry1Ab Bt maize: Field resistance, contributing factors and lessons from South Africa. *Crop Protection* 54: 154-160.
38 BusinessWeek (2014) Ware on cornfield pest sparks clash over insecticide. http://www.businessweek.com/news/2014-06-10/war-on-cornfield-pest-sparks-clash-over-insecticide. Accessed 10/1/2105.
39 Tabashnik, B.E., Gassmann, A.J., Crowder, D.W. & Carriére. Y. 2008. Insect resistance to Bt crops: evidence versus theory. *Nature Biotechnology* 26:199–202.
40 Monsanto (n.d.) *Monsanto Today: for the record: India pink bollworm*. www.monsanto.com/monsanto_today/for_the_recor/india_pink_bollworm.asp. Accessed 10/1/2015.
41 Zhang, H., Yin, W., Zhao, J., Jin, L., Yang, Y., Wu, S., Tabashnik, B.E. & Wu, Y. 2011. Early warning of cotton bollworm resistance associated with intensive planting of Bt cotton in China. *PLoS One* 6(8): 615–624.
42 Vachon, V., Laprade, R. & Schwartz, J.L. 2012. Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: A critical review. *Journal of Invertebrate Pathology* 111(1):1-12.
43 Van Frankenhuyzen, K. 2013. Cross-order and cross phylum activity of *Bacillus thuringiensis* pesticidal proteins. *Journal of Invertebrate Pathology* 114:76-85.
44 Dawn.com (n.d.) *Hidden potential for cowpeas*. http://www.dawn.com/news/225975/hidden-potential-of-cowpeas. Accessed on 10/1/12015.
45 Schmidt, J.E.U., Braun, C.U., Whitehouse L.P. & Hilbeck A. 2009. Effects of activated Bt transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird Adalia bipunctata in laboratory ecotoxicity testing. *Arch Environ Contam Toxicol*. 56(2): 221–228.
46 Hilbeck, A., W. Moar, M. Pusztai-Carey, A. Filipini & F. Bigler 1998. Toxicity of Bacillus thuringiensis Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* 27(5): 1255-1263.
47 Bøhn, T., Primicerio, R., Hessen D.O. & Traavik, T. 2008. Reduced Fitness of Daphnia magna Fed a Bt-Transgenic Maize Variety. *Archives of Environmental Contamination and Toxicology* 55(4): 584-92.
48 Zwahlen, C., Hilbeck, A., Howald, R. & Nentwig, W. 2003. Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris. Mol Ecol.* 12(4):1077-86.
49 Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C. & Griffiths, N.A. 2007. Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *Proceedings of the National Academy of Sciences of United States of America (PNAS*), 104:16204-16208.
50 Wu, K., Li, W., Feng, H. & Guo, Y. 2002. Seasonal abundance of the mirids, Lygus lucorum and Adelphocoris spp. (Hemiptera: Miridae) on Bt cotton in northern China. *Crop Protection* 21:997-1002.
51 Lu, Y., Wu, K., Jiang, Y., Xia, B., Li, P., Feng, H., Wyckhuys, K.A.G. & Guo, Y. 2010. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* 328, 5982: 1151-1154.
52 Men, X., Ge, F., Edwards, C.A. & Yardim, E.N. 2005. The influence of pesticide applications on *Helicoverpa armigera* Hübner and sucking pests in transgenic Bt cotton and non-transgenic cotton in China. *Crop Protection* 24: 319-324.
53 EENEWS.NET (2010) Greenwire. www.eenews.net/public/Greenwire/2010/05/17/3. Accessed 1/1/2015.
54 Tamò, M. & Srinivasan, R. 2012. *Biological control: new cure for an old problem*. CGIAR SP-IPM Technical Innovation Brief No. 18, August 2012.

55 Kabambe, V.H., Mazuma, E.D.L., Bokosi, J. & Kazira, E. 2014. Release of cowpea line IT99K-494-6 for yield and resistance to the parasitic weed, *Alectra vogelii* Benth. in Malawi. *African Journal of Plant Scienc* 8(4):196-203.
56 http://www.sneb.org/2014/SNEB%20presentation_Lungu.pdf
57 ICRISAT. 2013. *Bulletin of tropical legumes.* http://www.icrisat.org/tropicallegumesII/pdfs/November-2013.pdf. Accessed on 10/1/2015.
58 Lungu, E. *Development of weaning diets using locally available foods to combat malnutrition and food insecurity in Malawi*. http://www.ccrp.org/sites/default/files/cowpea_resistance_to_alectra___year_1__development_and_promotion_of_alectra-resistant_cowpea_cultivars_for_smallholder_farmers_in_tanzania_and_malawi.pdf. Accessed 19/02/2015.
59 Wood, L. & Moriniere, L. 2013. *Malawi climate change vulnerability assessment*. Nairobi: United States Agency for International Development. http://community.eldis.org/.5b9bfce3/Malawi%20VAFinal%20Report_12Sep13_FINAL.pdf. Accessed 17/02/2015)
60 Mid-term review of Programme for Africa's Seed Systems: Malawi – June 2010.
61 http://www.pecad.fas.usda.gov/cropexplorer/al/malawi_economy.pdf
62 http://www.asti.cgiar.org/diiva/malawi/cowpeas; (accessed 16/02/2015)
63 African Centre for Biosafety 2014. *Running to stand still: Small-scale farmers and the Green Revolution in Malawi*. Johannesburg: African Centre for Biosafety.
64 Chirwa, E. & Dorward, A. 2013. *Agricultural input subsidies: The recent Malawi experience.* Oxford: Oxford University Press.
65 Integrated Seed Sector Development (ISSD). 2012. *Malawi Seed Sector Assessment*. ISSD Briefing note – September 2012.
66 http://www.esfim.org/wp-content/uploads/ASSMAG-seed-systems-study.pdf
67 Seed Trade Association of Malawi 2014. *Malawi Seed Industry*. Presentation given at Bunda College of Agriculture, 8th April 2014. http://aginnovation.org/malawi/workshop/Malawi-Seed-Industry_Mr.%20Supply%20Chisi.pdf. Accessed 16/02/2015.
68 Mbwaga, A.M., Kabambe, V. & Riches, C (eds). 2008. *Development and promotion of Alectra resistant cowpea cultivars for smallholder farmers in Malawi and Tanzania*. McKnight Foundation Collaborative Crops Research Project No: 06-741. http://www.ccrp.org/sites/default/files/cowpea_resistance_to_alectra___year_2__development_and_promotion_of_alectra-resistant_cowpea_cultivars_for_smallholder_farmers_in_tanzania_and_malawi.pdf. Accessed 16/02/2015.
69 African Centre for Biosafety 2014. *Running to stand still: Small-scale farmers and the Green Revolution in Malawi*. Johannesburg: African Centre for Biosafety.
70 Hella, J.P., Chilongo, T., Mbwag, A.M., Bokosi, J., Kabambe, V., Riches, C. & Massawe, C.L. 2013. Participatory market-led cowpea breeding in sub-Saharan Africa: Evidence pathway from Malawi and Tanzania. *Merit Research Journal of Agricultural Science and Soil Sciences* 1(2): 011-018.
71 FEWS NET 2007. *Cross border trade update*. Ahttp://www.fews.net/sites/default/files/documents/reports/Malawi%20Informal%20Cross-border%20Trade%20Update%20December%202007.pdf. Accessed 16/02/2015.
72 FEWS NET 2012. *Informal cross border food trade in Southern Africa*. http://www.fews.net/sites/default/files/documents/reports/Southern%20Africa%20Informal%20Cross-Border%20Food%20Trade%20Bulletin%20-%20August%202012.pdf. Accessed 16/02/2015.
73 Bøhn, T., Aheto, D.W., Mwangala, F.S., Bones, I.L., Simoloka, C., Mbeule, I., Wikmark, O.-G., Schmidt, G. & Chapela, I. 2013. Co-existence challenges in small-scale farming when farmers share and save seeds. In Breckling, B. & Verhoeven, R. 2013. GM-Crop Cultivation – Ecological Effects on a Landscape Scale. *Theorie in der Ökologie* 17. Frankfurt: Peter Lang.