The Impact of *Agrobacterium tumefaciens* and other soil borne diseases on productivity of roses in East African region

An issue paper prepared by

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Executive summary

Crown gall is caused by *Agrobacterium tumefaciens*, a Gram-negative bacterium that is normally associated with the roots and crowns of many plants including roses, grape vines, stone fruits, nut trees, sugar beets, horse radish, rhubarb and others. The bacterium can survive in soils with good aeration such as sandy loams where diseased plants with crown gall have been grown. The bacteria enter the plants mainly through wounds and the higher the inoculum the bigger the galls and the severe the infection. The disease is characterized by a proliferation of cells on many parts of susceptible plants especially at the crown and roots resulting in big swellings known as galls hence the disease name crown gall. Losses range from 5 to 60% depending on age and variety.

Crown gall being a bacterial disease is difficult to control as the effective chemicals are antibiotics which are also used for management of human and animal diseases. Although a number of pest control products have been tested locally for registration purposes, none has been found to be effective for control and management of crown gall in Kenya. Use of non-pathogenic strains of *Agrobacterium radiobacter* has played a major role in the management of crown gall in Australia and USA. The non-pathogenic strains of *Agrobacterium radiobacter* have the ability to compete with pathogenic strains for food and space and also produce an antibacterial compound that inhibit growth of the pathogen. Many growers try to manage the disease by using clean planting materials and when the disease appears they coat the affected areas with copper fungicides, vegetable or petroleum oils after plucking the galls. The selection of pest and disease free seeds, plants and cuttings is very important. All plants brought into the nursery should be carefully inspected and infected planting material discarded and destroyed. It is important that tools used for pruning, and harvesting rose stems should be disinfected between cuts to avoid spread from one plant to another.

In the East African Community Agriculture and Rural development policy, partner states committed to establish and coordinate mechanisms for monitoring and surveillance of transboundary pests and diseases. In order to manage diseases and pest, governments have put in place national policies, laws and subsidiary legislation in the form of regulations. In Kenya for instance a number of statutes have been enacted such as the Agriculture Act, The Plant Protection Act, the Seed and Plant Varieties Act, the Pest Control Products Act. Importation of vegetative parts of roses for propagation requires that such material is imported through Plant Quarantine arrangement. This is critical to prevent introduction of more virulent strains of *A. tumefaciens*. Private industry standards such as the Kenya Flower Council Code of Practice, have also been introduced to help in Management of diseases among other objectives. Despite all the efforts, Crown gall still remains a major challenge in the production of roses in East Africa.

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Background – Horticulture In Eastern Africa

Horticulture is an important sub-sector of E. African agriculture in achieving food security, income and employment generation, foreign exchange earnings, raw material for agro-processing, and poverty alleviation.

In Kenya the horticulture sub-sector contributes 33 percent of agriculture's share of the Gross Domestic Product. The domestic value of horticultural produce is estimated at Kshs. 153 Billion. The sub-sector employs over 6.5 Million workers directly and indirectly. In 2010, the sub-sector was second to tea with a foreign exchange earning of Kshs. 77.7 Billion from horticultural exports. Earnings from flower exports over the same were Kshs. 35.5 Billion. Kenya's horticultural exports grew at an average annual rate of 15.9 percent between 2001 and 2010. (Ministry of Agriculture 2010). Kenyan flowers account for 30 - 34% of flowers auctioned in Europe. Roses make up 74% of Kenya's flower exports. In 2010, Kenya exported to Europe 120,220 Metric Tonnes of flowers, (KFC personal communication). It is estimated by 2010, the flower industry was providing direct employment to over 90,000 and over 500,000, in related industries (Arim, 2011).

In Uganda the flower industry started in 1993 with a lone 2 hectare commercial farm exporting 400 tons worth less than $5m to 20 farms covering over 192 ha of land by 2009. By 2005 the flower industry had grown with farms producing up to 6000.tons of flowers worth US$32 million (Van der Hulst 2005). It is the 8th most important export earner for the country and the 3rd most important non-traditional export revenue source. The sector now directly employs 6,000 persons while an additional 30000 persons earn a living indirectly from the flower sector (Levitt, 2011). Floriculture consumers demand for high quality flowers free of damage caused by pests and diseases and produced in an environmentally friendly manner (Pizano, 2001).

Production of flowers and cuttings for export is a relatively new sector in Ethiopia and is seen as having good potential to contribute to poverty alleviation and economic development through the creation of employment and earning of foreign exchange. The Government of Ethiopia has provided an attractive package to encourage investment in the sector. Since 2002 the sector has grown at an amazing rate and now comprises more than 1,000 ha of greenhouse production that provides employment for approximately 50,000 people and export earnings of US$ 127 million (Humphries, 2011). Many Ethiopians not directly involved with flowers are also benefiting from the sector (Masila and Mikael, 2008).

In Tanzania the flower industry was established in the mid 1980s. The sector has registered tremendous growth in the past three years most of which is attributed to the presence of the vibrant TAHA. Currently the export sub-sector earns the country more than USD 340 million of foreign income and has registered a growth rate of 8-10% per annum. The sector is therefore recognized as an engine for country's socio-economic growth and a significant contributor in the poverty alleviation mainly in the rural areas.

Agrobacterium Tumefaciens And Other Soil Borne Diseases

Soil borne organisms such as plant parasitic nematodes, fungi, bacteria, mycoplasma like organisms, and viruses are among the most underestimated of the factors which affect plant productivity in tropical regions. Other soil borne disease causing agents of economical importance in Kenya are *Fusarium oxysporium f. sp. Rosae*, Pythium, Rhizoctonia and Nematodes specially the *Meloidogyne hapla*. These pests are relatively easier to manage compared to *Agrobacterium radiobacter*; and are managed mainly through use of recommended fumigation practices and application of fungicides.

In Ethiopia soil borne fungi and nematodes, have no significant economic importance in the flower producing sector. However, it appears that the only soil-borne pathogen of economic importance in commercial greenhouse flower sector in the country is the crown gall forming bacteria, *Agrobacterium tumefaciens* (Derso and Yalemtesfa 2011)

The most common diseases and pests affecting the flowers in Uganda are crown gall of roses and nematodes (Pizano and Lorenzo, 2001). *Phytophthora species* are root pathogens of roses and cause brown root rot. The aboveground symptoms are initially characterized by yellowing, browning and defoliation of some of the lower leaves, followed by reduced plant growth, dwarfed leaves and shoots and interveinal chlorosis of the younger leaves (Armsing, 1995).

Nematodes are the most notorious soil pests for the floricultural industry in Tanzania. The problem is experienced by both categories of flower farming systems (hydroponics and direct soil media) and the industry has struggled with the control of nematodes for period as long as the history of the industry. The nematodes whose main effect is stunted growth and pale green leaves in roses also affect other flower varieties like the chrysanthemums.

It has been reported that some of the farms in Tanzania with their total production on non-soil media and hydroponics have managed a zero tolerance on soil-borne diseases. They do not have any cases of soil-borne diseases and have continued managing the diseases by using preventive measures.

Rose plants infected by *Agrobacterium* manifest slower growth, stunting, yellowing and chlorotic leaves and fail to produce healthy flowers. The severely infected plants develop sensitivity to environmental stress. The pathogen can survive in the soil for more than ten years. Severe infection kills the plant. Roses that have crown gall develop rounded tumours which are brown to brownish black in colour, and develop from smooth spongy to rough texture with age. Expanding tumours destroy adjacent healthy tissue and prevent normal flow of water and nutrients.

Other soil borne pathogens causing diseases of economic importance in flowers include; *Fusarium, Pythium, Rhizoctonia, nematodes etc.*

What is *Agrobacterium*?

Agrobacterium tumefaciens is an aerobic gram negative rod shaped and motile non sporing bacteria. The motility is due to the flexuous peritrichous flagella on the cell body. It has circular chromosome with two plasmids one linear and the other one circular. It is a soil inhabiting bacterium found mainly in the rhizosphere. It is known for its disease inducing ability on many plants. The disease is characterized by a proliferation of cells on many parts of susceptible plants especially at the stem base and roots resulting in big swellings known as galls hence the disease name crown gall. The cause of the swellings is the presence of plasmids that have the ability to induce tumours when taken up by a susceptible host. Plasmids are non-nuclear extrachromosomal DNA found in the cytoplasm and which can be transferred to other cells by conjugation. The plasmids containing DNA with the ability to cause cells to proliferate and form tumours are known as Ti (Tumour inducing) plasmids. They are approximately 200 kilobases (Kado 2002).

The bacterium is also known for its ability to transform other living cells by the transference of part of its DNA from its plasmid called T-DNA. The T- DNA can be used to attach other genes of interest from other sources which are co transferred with the T-DNA. These genes and the T-DNA get incorporated in the nuclear DNA and expressed by the recipient host. The DNA transmission capabilities of *Agrobacterium* have been extensively exploited in biotechnology as a means of inserting foreign genes into plants following the discovery of gene transfer mechanism between *Agrobacterium* and plants. This resulted in the advancement of methods to alter the bacterium into an efficient delivery system for the development of transgenic plants and a useful tool in transformation studies. Besides bacteria mediated transformations other methods are also used and together have developed a whole discipline of genetic engineering.

Crown gall

Crown gall is the name of the disease caused by *A. tumefaciens*. It produces galls on the roots and at the crown of woody plants. The disease affects many plant species belonging to over 93 plant families (Kado 2002). Apples, pears, cherries, apricots, grapes and ornamentals like roses and chrysanthemums are affected. Vegetables like tomatoes and sweet pepper are also affected. The disease causes economic losses on susceptible crops. *Agrobacterium tumefaciens* can move systemically throughout the root system and can wipe out a crop.

The galls develop due to interference by growth hormones, the auxins and cytokinins produced by the infective *A. tumefaciens* once inside the host cell. The infected host cell proliferate uncontrollably at the expense of the host. The bacteria use the host systems to produce compounds for their growth thus keeping them active in the host.

Protective measures by use of stringent hygiene practices are recommended especially in high value crops like roses. Many other control measures are being sought especially biological control using non pathogenic stains of *Agrobacterium*. The issue of resistance development is pertinent and is dealt with by use of genetically modified strains. This is only allowed in some countries like USA and Australia.

Fig. 1. Rose plants showing crown gall at the crown and on young stems (courtesy E. Mutitu and P. Ngaruiya).

Pathogenesis

The strains of the bacterium are divided into three biotypes 1,2 and 3. Biotype 1 has a wide host range and is the most common.

The bacterium is soil borne and can survive on the root surfaces of many alternate hosts such as weeds. In the soil the motile cells respond to the root exudates, the sugars and the amino acids especially where the plant tissues have been wounded. Other compounds released by the wounds are phenolic compounds like acetosyringone which strongly attract the bacteria and they move towards the plant surface. The compounds also activate the virulence genes (Vir genes) on the Ti plasmid. Once the bacteria reach the plant surface they release the T-DNA which enters the plant cells and gets incorporated into the nuclear DNA. Once part of the plant genome it codes for the production of cytokinins, indoleacetic acid and the synthesis of compounds known as opines and agrocinopines (Deacon *et al* 2006).

The hormones are the ones that bring about the imbalances that lead to the production of galls. The opines are amino acids derivatives and the agrocinopines are phosphorylated sugar derivatives utilized by the *A. tumefaciens* as the sole source of carbon. The bacterium multiplies as it is favoured by the conditions at the expense of the plant host.

Infected plants may appear stunted with smaller foliage and blooms, and generally seem less healthy. This is caused by the gall cells growing and damaging the tissues of the plant such as the xylem and phloem vessels, and reducing the amount of nutrients and water that can be transported through the plant.

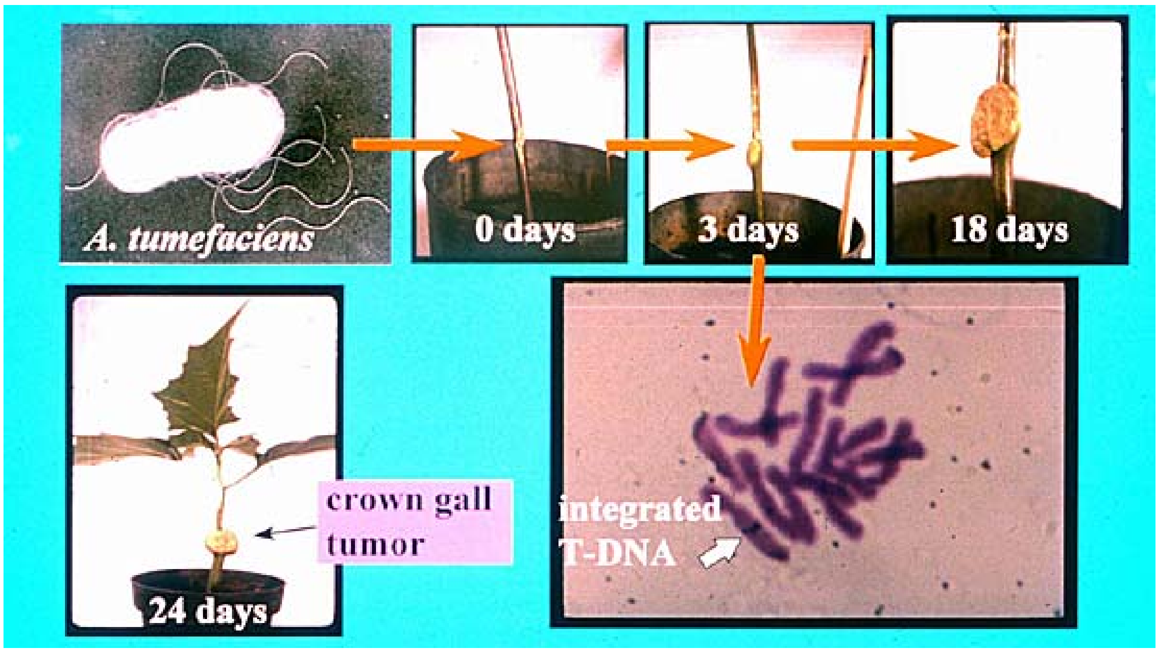
It is important to remember that about 90% of *Agrobacterium* in the soil are non pathogenic and normally referred to as *A. rhizobacter*. It is closely related to Rhizobium bacteria that live symbiotically with a number of leguminous plants forming nodules that synthesize nitrogen for the host plants. Many Rhizobia are also non infective and do not produce nodules but are soil inhabitants.

Disease cycle

Infection starts from infected soil where a susceptible host is planted. Other sources of inoculum are irrigation water, infected planting materials, pruning equipment, cultivation equipment and rouged plants and detached or disintegrated galls put back in the soil.

The bacteria enter the plants through wounds and the higher the inoculum the bigger the galls and the severe the infection. Infection can be haboured by the plant for a long time without symptoms only to appear later when the plant is wounded.

Once the bacteria enter the wounds into the plant it takes about two weeks for the galls to start appearing. The gall cells are not protected by an outer epidermal layer and with time they start cracking and become brittle and start disintegrating. Old galls darken and look rugged and sometimes become infested with insects that feed on the cells. Eventually they fall off back into the soil and are released to start the infection cycle once a host is replanted and the conducive environment of wounds occur



Development of crown gall *Datura stramonium* experimentally inoculated with *A. tumefaciens* after 2-3 weeks, (Courtesy C. Kado, 2002)

Crown gall on roses in Eastern Africa

Crown gall is widespread in rose farms and nurseries in Kenya. Growers say it is present in every rose farm at varied incidence levels of 10 to 15%, with certain varieties being more susceptible (Pers. Comm.). In Kenya, the disease prevalence was noticed in 1998, when many flower farms that went into commercial production of roses were severely affected by the outbreak of the disease. The disease is believed to have been introduced in Kenya through infected root stock of roses imported from Israel. Infected fields are reported to have a short lifespan that is not economical. Failure to observe strict nursery hygiene practices leads to spread to other rose farms. In the past some nurseries closed shop due to widespread infection of their nurseries.

It is reported that losses range from 5 to 60% depending on age and variety (Real IPM 2007). A survey done by Weller in 2006 found six out of 400 isolates of *A. tumefaciens* isolated from East African rose samples were pathogenic (Real IPM 2007).

Affected rose plants become stunted produce fewer marketable stems and generally lack the vigour of healthy roses. The bacterium affects production through reduced number of good quality stems, reduced length of harvestable stems and production lifespan. Some growers do not exercise good sanitation practices and drop pruned infected branches on the ground where the bacteria find their way back to the soil ready to infect again.

Disinfection of pruning tools is not strictly followed all the time in some farms and this assists the spread of the disease within the farms. A number of farms have made arrangements with the rose breeders to propagate their roses and where good hygiene is practiced they manage to keep the disease under check. It has been observed that Roses grown on both soil and substrate like pumice and coco peat get infected but the soil grown roses are more severely affected.

In early 2000, Melissa flowers in Uganda had to replace 1.5ha (three greenhouses) of Golden gate, Sunbeam and Jupiter varieties because they were affected by the disease. (Eboku,*et al*, 2011). Due to susceptibility of some varieties, the farms have to be selective on the varieties to grow and those to avoid. Small but costly trial units must be run to evaluate and select the most appropriate varieties before large scale planting could be done. This has denied some farms the opportunity of growing some quite productive and competitive varieties. Additionally farmers in such circumstances lose customers to other competing producers or even countries. In general, there is inadequate assessment and documentation of the actual socioeconomic impact of crown gall in Uganda. There is a need for studies to estimate impact and to develop and disseminate appropriate technologies for control of these diseases in the flower sector.

Correlation between climate and disease

Plant disease is influenced by three major factors, namely susceptible host, virulent pathogen and conducive environment. Increase in moisture enhances the risk from pathogen infection while rise in temperature favours some pathogens. This is not universal as some hosts become more resistant to infections with temperature rise (Coakley *et al* 1999). Some bacteria are known to infect more severely at cooler temperatures like halo blight of beans caused by *Pseudomonas phaseolicola.*

Crown gall on various hosts is known to be influenced by factors such as location of galls, cultural practices, cultivars and most important climate. The disease is most common and severe in cold climates where freeze injury occurs. Crown gall on grapes has been reported to be severe where winter injuries create avenues for *Agrobacterium* entry into the host. The bacterium also survives systemically on the host and infects when conducive environment appears. Heat is able to suppress crown gall and hot water treatment of infected planting materials reduces the disease.

Disease infection levels

Crown gall disease levels vary depending on variety and age of the crop. The actual level of infection in the Eastern African region is not documented. Losses of 5-60% have been reported in Kenya (Real IPM 2007). Infection of up to 100% has been reported on grapes in cold climates where winter injuries occurs. Infected planting materials lead to severe disease and heavy losses are incurred. The crop does not last for the recommended period of growth due to severe infection. The disease even spreads to the rose branches.

Innovative Research and Development

A limited number of studies on managing *Agrobacterium tumefaciens* have been conducted in Kenya. A research on a proper accurate testing, description of infection, and agents that control the bacterium with the Ti-plasmid was undertaken by “Kreative Roses”, the Kordes Production Company at Lake Naivasha in collaboration NAK Tuinbouw in Netherlands, and FERA (formerly CSL) in the UK. The research did not provide conclusive results due to lack of accessibility to a PCR machine at that time (Arim 2011).

Research on production of *Agrobacterium* free nursery stocks using biological control focusing on use of *A. radiobacter* strains that attack Ti *Agrobacterium* in combination with use of beneficial fungus was undertaken jointly by FERA and Real IPM. This research was anticipated to develop a certification scheme for ensuring *Agrobacterium* free nursery free stock. The research aimed at detecting infection at the latent stage other than using symptom for diagnosis. The research was funded by DFID and African Challenge fund. They also lacked the necessary facilities for diagnosis and therefore they could not come up with conclusive results.

A great deal of research is on going in *Agrobacterium tumefaciens* in transformation and related molecular work. The genome has been sequenced. As for work in plant disease, biological control using non pathogenic strains of *Agrobacterium radiobacter* is going on. Genetically modified strains where resistance genes are deleted like in the strain K1026 are registered for use in countries like USA under the trade name Galltrol.

Work on growth promoting bacteria has shown that they inhibit tumour development in plants. This is due to the work of certain enzymes that lower ethylene levels leading to reduced tumour growth.(Hao *et al* 2007). Further work on Vir- gene encoded T-DNA transfer process is ongoing. Work on identification of plant genes involved in *A.tumefaciens* transformation continues (Escobar and Dandekar 2003).

A number of research have been undertaken to elucidate management or control of nematodes (*Meloidogyne hapla*), Fusarium (*Fusarium oxysporium f. sp. Rosae*), Pythium, and Rhizoctonia in production of roses. Currently, there is on-going research on management of *Fusarium oxysporum f.sp. rosae*, *Meloidogyne* sp., and weeds using chemicals and Brassica biofumigants in greenhouse rose production (*Rosa* sp.) at Egerton University. Another research currently on-going at the University is on management of Root Knot Nematodes and weeds using different amendments in greenhouse growing *Asclepias tuberosa* L.

Effective management of soil borne pathogens in high value crops like cut flowers used to be by fumigation using methyl bromide. When Methyl bromide was listed as an ozone depleting substance in the early 1990s, countries were mandated to phase it out before 2015. A number of alternatives were successfully tested and adopted in Kenya and growers are currently using them in place of methyl bromide. These methods include, substrate culture using locally available materials like pumice (volcanic rock mined in Naivasha-Rift valley), coco peat (coconut byproduct), steam, metham sodium, and for small scale farms, use of good water management systems like gravity drip irrigation coupled with seed treatment was very successful.

Experiences in cultural, chemical and biological interventions

Cultural methods

Plant nursery disease management programs should be designed to maintain healthy plants. The first steps in a pest management program are preventative, starting with clean plant production nursery area (Good Agricultural Practice Bulletin, 2007). Proper site selection of plant nurseries is very critical. Plant nurseries should be cleared of previous crop debris prior to establishing a new crop to help eliminate pest problems from previous crops. Weed control in and around plant nursery areas eliminates alternate hosts. The selection of pest and disease free seeds, plants and cuttings is very important. All plants brought into the nursery should be carefully inspected and infected planting material discarded. The key to producing clean planting material includes monitoring, scouting and recording of pests and diseases present. Other cultural considerations include: avoid damaging roots and stems whenever handling the plants, use clean tools or hands when pruning and disinfect regularly and rogue all infected plants. It is also important to treat irrigation water, particularly recycled water to kill microorganisms.

Some growers use pest-resistant or tolerant plants to reduce the need for pesticides. Some reported that some varieties are more susceptible to crown gall than others e.g "Wild thing" is very susceptible while "Red calypso" can survive an attack by *Agrobacterium* (Pers. Commun).

Control of root chewing insects and piercing nematodes is vital in managing the bacteria as it mainly enters the plant through wounds. Plants infected should be rouged as a matter of routine practice (Sigee, 1993; Ryder & Jones 1990). Other growers practices reported to reduce infection of *A. tumefaciens* include incorporation of high organic matter content in crop land and regulating nutrients to promote uptake of copper.

Water can harbour *Agrobacterium tumefaciens* and should therefore be treated before use for irrigation. A water source situated close to an infected nursery could become contaminated and thus serve as a source of inoculum for further infection.

In an effort to reduce yield losses caused by Crown gall, growers have been using various unconfirmed methods of disease control. This is due to lack of registered products for control of crown gall. Gall removal with concomitant application of disinfecting paste may prevent the gall regeneration at the treated site but will not stop systemic spread of the pathogen within the plant and further gall formation. There is a common belief that application of bactericidal pastes controls the disease.

Since the pathogen moves systemically in plants, it is important that tools used to cut plants should be disinfected between cuts to avoid its spread from one plant to another. Sodium hypochlorite (NaOCl) or Jik (household bleach) is an effective disinfectant to use for this purpose. A dilution of about 1% NaOCl in water is effective against the bacterium.

The dressing of galls directly with a mixture 2, 4 – Xylenol and metacresol in an oil-water emulsion has also been reported in Kenya as effective in managing established tumours. Some farmers in Tanzania have also resorted to the use of olive oil as a control measure. The olive oil is applied on the galls to prevent the further growth.

A discussion with some of the main growers revealed the use of the following:

Oserian

1. Corn oil eg Elianto, Fresh fry is applied on infected plant parts. The galls are cut then pasted.
2. Olive oil—applied on infected plant parts after the galls are cut.
3. Copper based fungicides such as Kocide applied neat.

Finlays

Crown gall is management is done by sterilizing the roll cut using Didecyldimethyl Ammonium chloride (120g/L) at 2 ml / L.

Fresh fri cooking oil is also being used in the management of crown gall. The galls are cut then pasted with Fresh fri which suffocates the gall. In house trials still ongoing but results are encouraging.

Other farms

The infected part is cut and Copper based fungicides mixture with petroleum gel and applied on the wound. This is said to reduce the effects of the disease on the crop. Copper based products are reported to be effective but can be phytotoxic if sprayed directly at the same concentration on the foliage. Spore kill is also used to disinfect cutting tools.

Biological control

In general, bacterial diseases of plants are very difficult to control owing to the lack of effective chemicals. Antibiotics could be used, but they are useful for treatment of human diseases and are not generally allowed for use in agriculture. Kerr discovered that non-pathogenic strains of *Agrobacterium radiobacter* have the ability to compete with pathogenic strains for food and space in mixed inoculations, preventing the pathogenic bacterium from becoming established (Farrand 1990). There is also evidence that *A. radiobacter* produces an antibacterial compound that inhibit growth of the pathogen. *A. radiobacter* strain K84**,** completely prevented disease when added to wound sites at a 1:1 ratio with cells of *A. tumefaciens*. This strain is marketed in many parts of the world and it is used by suspending the bacterial cells in water, then dipping seeds, seedlings or cuttings in this suspension before planting. One disadvantage is that it acts only as a preventative treatment, and not curative. K84 inhibits strains of *A. tumefaciens* containing a nopaline-type T_i plasmid which are also the most common strains attacking horticultural crops. Some resistance to *A. radiobacter* strain K84 has been reported to occur due to the transfer of the genes responsible for conferring immunity to agrocin 84 antibiotic (Real IPM, 2007). A genetic modification by deletion of the gene responsible for exchange of DNA material among bacteria has resulted to the development of a new strain, *A radiobacter*, strain K1026 in Australia (Manual of Biocontrol agents, 2004). This strain is not capable of conferring resistance to other agrobacterium that are sensitive to the agrocin antibiotic.

A trial carried out to test *Agrobacterium radiobacter* strain K84 as a potential biocontrol agent against Crown gall in Commercial Flower Farms in Ethiopia showed that there were no significant differences ($P \leq 0.05$) in the number of galls between treated and untreated plots at one flower farm, while significant differences ($P \geq 0.05$) were observed in two farms (Derso and Yalemtesfa 2011). Significant yield increase ($P \geq 0.05$) on rose yield between treated and untreated plots were observed at all locations. Significant differences ($P \geq 0.05$) in stem diameter between treated and untreated plots were observed at Oromia Wonder, but not at Joy Tech and ENYI. The results showed *A. radiobacter* strain *K84* to be effective in preventing crown gall and reduced the losses, however, it must be used as a component of integrated disease management.

Chemical control

There are no effective chemical controls for crown gall. Although antibiotics and copper bactericides are able to kill the bacterium on contact, they do not penetrate the plants and therefore fail to come into contact with bacteria residing systemically (*Burr 2004).*

Other soil borne diseases

The control of Fusarium (*Fusarium oxysporium f. sp. Rosae*), Pythium, and Rhizoctonia is mainly through use of recommended fumigation practices and application of fungicides such as Tolclofos Methyl, Propamocarb Hydrochloride, Carbendazim and Pentachloroni-Trobenzene. Use of fungi that form beneficial mycorrizae including Arbscular mycorrhizae has also been reported to be effective in fungal associated problems in production of roses. Other practices adopted by farmers include use of resistant / tolerant varieties and use of clean planting materials. In addition, use of UV/UF machines in water treatment for each of these soil borne diseases is becoming popular among growers.

Metam sodium is registered for use as a general Pre-plant soil disinfectant for control of soil borne pests (root-knot nematodes, bacterial wilt).

Regional policies and action on disease control systems

Activities to develop regional approaches to disease and pest management have been ongoing for the last one decade. In November 1999, the Treaty for the establishment of the East African Community was signed by the three heads of state- Kenya, Uganda and Tanzania. Article 108 of the treaty focuses on Plant and animal Disease Control and states that Partners states shall:

a) Harmonize policies, legislation and regulation for enforcement of pest and disease control.

b) Harmonize and strengthen regulatory institutions.

c) Adopt common mechanism to ensure safety, efficacy and potency of agricultural inputs including chemicals, drugs and vaccines etc.

In the East African Community Agriculture and Rural development policy, partner states committed to establish and coordinate mechanisms for monitoring and surveillance of transboundary pests and diseases, and to promote pest and disease management programmes in order to reduce the impact of pest and diseases. In an effort to provide for efficient and effective sanitary and phytosanitary regulatory and control measures among partner states, the East African community has developed a protocol for sanitary and phtosanitary measures which will be used as a the key guiding document in the region. Other documents that have been developed include: Harmonized application forms for registration of pest control products, Labelling requirements and procedures for testing efficacy of pest control products for on plants. It is hoped that once the policy documents are implemented partner states will be equipped to face the main challenges facing growers, including Crown gall challenge as a block.

National policies and action on disease control systems

A number of general national policies have been in place in order to address challenges facing agricultural productivity. These include; Strategy for Revitalization of Agriculture and the current Agricultural Sector Development Strategy. In order to manage diseases and pest, governments have put in place national policies, laws and subsidiary legislation in the form of regulations.

Vision 2030 has identified agriculture as one of the key sectors to deliver the 10 per cent annual economic growth rate envisaged under the economic pillar. Pests and diseases such as crown gall can threaten access to existing market. Vision 2030 calls for proactive efforts to maintain existing markets and create new ones to increase Kenya's bargaining power in global agricultural markets.

Pest and disease control in crops has been identified in the Agriculture Sector Development Strategy (ASDS) as a major challenge to most farmers, especially small- and medium-scale operators, due to high cost of pesticides and control equipment. Although the ASDS does not single out crown gall of roses, the strategy recognizes that there has been high levels of waste due to pre- and post-harvest losses occasioned by pests and diseases, and lack of proper handling and storage facilities. Smallholder farmers are unable to control pests and diseases due mainly to lack of information. Poor access to agricultural information and technologies, limited access to markets and narrow market destinations for various commodities have been identified as other challenges.

The Agriculture Act Cap 318 was enacted in 1955 to promote and maintain a stable agriculture, to provide for the conservation of the soil and its fertility and to stimulate the development of agricultural land in accordance with the accepted practices of good land management and good husbandry. Section 191 empowers the minister to declare a crop to be a special crop and to establish an Authority for promoting and fostering the development of special crops. Through such powers the Kenyan Government established the Horticultural Crops Development Authority (HCDA) with the mandate of promoting development of horticultural crops. HCDA is also mandated to address regulatory issues on establishment and management of nurseries; purchase and sale of seed and seedlings; control and supervision of cultivation of crops, inspection of growing and harvested crops among others. Section 204 empowers any authorized officer at all reasonable times to enter any building or structure on any land and examine and inspect or crops or other produce.

The Plant protection Act prohibits importation of plant, plant product and regulated article unless there is an import permit granted by the National Plant Protection Organization or an original Phytosanitary Certificate issued by National Plant Protection Organization of the exporting country. The Act also makes it mandatory for importers to report the arrival of any consignment of plant, plant product or regulated article and directs the importer to apply for phytosanitary inspection to a plant inspector in charge of the point of entry. It also empowers the inspector to take samples for laboratory tests and undertake follow up inspection during active growth stages. Where a consignment does not meet all the phytosanitary requirements, that consignment shall be intercepted, seized, detained, treated, disposed or destroyed in accordance with the law.

Upon conducting a Pest Risk Analysis, where risks associated with importation of a plant, plant product or regulated article are minimal, the National Plant Protection Organization may grant importation permit. Where risks are substantially high, the National Plant Protection Organization may grant permit for importation under plant quarantine procedures. Where risks are very high, the National Plant Protection Organization will prohibit importation except for the purpose of restricted essential such as scientific research and experiment or education provided that such importation shall not present significant risks to agriculture or natural environment of Kenya and shall be subjected to quarantine procedures.

Any person importing seed shall, in addition to meeting the plant protection requirements comply with the requirements of the Seed and Plant Varieties Act. Importation of vegetative parts of roses for propagation requires that such material is imported through Plant Quarantine arrangement. This is critical to prevent introduction of more virulent strains of *A. tumefaciens*. The material should be free from all pathogenic viral, fungal, bacteria, nematodes, mycoplasma diseases and insect pests of roses. The roots should be free from soil, treated with an appropriate chemical before dispatch. The Act also empowers the Minister to make regulations for:- control of the production, processing, testing, certification and marketing of seeds; for preventing the sale of seeds which are deleterious; for preventing the spread of plant disease by the sale of seeds; and for requiring the treatment of seed by any specified means, for the control of plant disease.

The Pest Control Products (PCP) Act was enacted in 1983 to regulate the importation, exportation, manufacture, distribution and use of products used for the control of pests and of the organic functions of plants and animals and for connected purposes.

According to the various articles of the FAO International Code of Conduct on the Distribution and Use of Pesticides, (2005) respective Governments have the overall responsibility of regulating the availability, distribution and use of pesticides in their countries and ensuring the allocation of adequate resources for the mandate.

Efficacy evaluation of a pest control product is important because it enables the registration authorities to evaluate the benefits to be gained from new products and to weigh those benefits against potential hazards due to their introduction. Article 6.1.2 of the FAO International Code of Conduct on the Distribution and Use of Pesticides stipulates that Governments should strive to establish registration schemes and infrastructure under which products can be registered prior to domestic use. This is done through the Pest Control Products Board.

Section 4 of the Pest control products Act prohibits importation of pesticides unless the products are registered for use in Kenya. Every pest control product, distributed, sold, exposed, offered for sale or for research purposes must bear on the container thereof, an approved label in understandable English and Kiswahili.

In Tanzania, the Plant Health Services(PHS) acts as the National Plant Protection Organization (NPPO) for Tanzania. Apart from being in charge of phyto-sanitary inspections, the PHS inspectors also visit the farms to conduct field inspections and also offer some advice to the farmers. However, this mostly rotates around the phyto-sanitary requirements. The Tropical Pesticides Research Institute (TPRI) is the national organization charged with post entry quarantine inspection services and registration of plant protection products. They have the mandate to ensure that only clean planting materials are imported into Tanzania.

Effectiveness of regulators to control soil borne diseases

The management and control of various diseases is vested in a number of institutions. KEPHIS is mandated under the Plant protection Act to prevent introduction into the country any diseased plant material. KEPHIS is also mandated to carry out inspection of crops during production and before exportation and to issue phtosanitary certificates.

In 2006, the National Plant Protection organization KEPHIS organized a national workshop involving the various stakeholders to discuss the crown gall problem and recommend ways of addressing it effectively. This was an attempt to sensitize growers on the negative impact of crown gall.

On the other hand, the Pest Control Products Board is mandated to regulate the importation, exportation, manufacture, sale and use of pest control products in the country. As the regulators implement the legal provisions, they are faced with a number of challenges:

1) Inappropriate, outdated and fragmented legal and regulatory framework has been identified in the Agriculture Sector Development Strategy (ASDS) as one of the challenges facing the development of the agricultural sector. In order to strengthen regulatory services, the agricultural sector is committed to rationalize the regulatory bodies to achieve economies of scale, improve efficiency, quality and synergies while minimizing overlaps and duplication. Laws that relate to delivery of plant protection services will be reviewed and their enforcement enhanced. The Agriculture act is old and outdated and lists such crops as Wheat, Barley, Oats, Beans, Finger Millet, Sorghum, rice, Sunflower and Sugar-cane on the list of scheduled crops. The list excludes other important crops like roses.
2) Despite the presence of KEPHIS inspectors at the port of entry, some diseased plant materials like roses infected with crown gall find their way into the country. Others may be imported in small quantities in brief cases and may be used as an avenue of introduction of new diseases.
3) Applicants submit applications for introduction of new pest control products based on anticipated market returns and not based on the economic importance of emerging diseases or pests. As such new pests may continue to be a challenge to growers where it is seen to be a minor problem to the agrochemical industry.
4) After a new product is tested and registered for use in the country such uses are recommended on the label and on the list of registered products. The application rates are based on local biological efficacy trials on specific pest and crop. It is difficult for the regulators to monitor uses in all farms in the country. Some growers disregard the recommended uses and use the product for other unregistered uses. Such uses may lead to under dosing or overdosing which may lead to the development of resistance
5) During efficacy trials for products against crown gall, absence of galls, the number and sizes of galls would be used as a measure of efficacy. This ignores latent infections. Such parameters used for measuring efficacy of pest control products may not be adequate to demonstrate performance.
6) Some growers import plant materials or unregistered pesticides into the country using private flights. Such plant materials and pesticides have not been subjected to risk assessment.
7) Regulators may not have the technical capacity to conduct risk assessment for some new products. Such weaknesses may cause delay in introduction of a potentially good product.

8) Registrants overseas fail to submit comprehensive dossiers and submit summarized extracts which are not enough to conduct risk assessment.

9) Political influence may ignore technical advice.

Standards and guidelines

KFC has developed a Code of Practice that is fully benched marked to GlobalGAP. The code helps in Industry Self-regulation and to meet international standards. KFC Codes of Practice detail the Standards that are required from its members to achieve one of the standards. It operates at 2 levels, that is, Silver and Gold Certification Standards. The standard puts emphasis on training of farm workers on safe and effective use of pesticides, Good Agricultural Practices and crop protection methods which embrace integrated pest management. The Code supports compliance with National laws by complementing the regulatory requirements by the various statutes.

Trials on products for registration against crown gall

A number of pest control products have been tested locally for the control and management of crown gall. A product based on Bronopol 27%w/w was tested for the control of Crown gall in roses and other bacterial diseases in Kenya. Bronopol has both bactericidal and bacteristatic effects. It acts by oxidation of mercapto group of bacterial enzymes causing inhibition of dehydrogenase activity, resulting to irreversible membrane damage. It acts as an imunomodulator by modifying the immune systems of plants. It mimics the natural Systemic Activated Resistance (SAR) by changing the contents of the phenols, proteins, nitrogen and certain enzymes and makes the plants resist bacterial attack. It was introduced in 1964 as a preservative for cosmetics, pharmaceutical preparations and industrial water systems for control of bacterial growth (Pesticide Manual, 2006).

Efficacy trial results showed that Bronopol could control other bacterial diseases such as bacterial wilt in tomatoes and potatoes, halo blight in French beans and bacterial soft rot in brassicas and was registered as an agricultural bactericide for the control of bacterial wilt in tomatoes and potatoes, halo blight in French beans and bacterial soft rot in brassicas. *Preliminary results on Crown gall in roses have not shown effective control of the disease.*

Another trial was started in 2010 to test the effectiveness of 40% Cupric hydroxide (expressed as copper) against crown gall. Rose plants with galls were identified and the galls cut and pasted with various treatments. The number of galls were counted on a weekly basis. The trial is yet to be concluded.

A product based on a genetically modified *Agrobacterium radiobacter* was submitted in 2006. The genetic material in *Agrobacterium radiobacter* K 84 responsible for the transfer of agrocin 84 has been spliced to form a new strain K 1026. *Agrobacterium radiobacter* competes with the gall causing organism *A. tumefasciens* for invasion sites on damaged woody stems thereby preventing the pathogenic bacterium from becoming established. It also acts as an antagonist by producing antibacterial compounds that inhibit growth of *A. tumefasciens.*

Since the product was based on an organism whose genetic material had been engineered, it required clearance by the National Biosafety Authority before it is submitted to the Kenya Standing Technical Committee for Imports and Exports (KSTCIE) and to PCPB for registration. The Clearance from the National Biosafety Authority is still being awaited. *Agrobacterium radiobacter* is reported to be approved for use in USA (Manual of Biocontrol agents, 2004).

Currently, no product has been registered for control of crown gall in Kenya.

Questions raised during the Teleconference

1. A question was raised on resistance development of *A. tumefaciens* to *Agrobacterium radiobactor strain K84*.

   It was clarified that Strain K84 works on A. tumefaciens by producing an antibiotic called Agrocin. The Strain K84 is not itself affected by the antibiotic due to the presence of a gene that confers immunity to agrocin antibiotic. This gene has been reported to be transferred to the A. tumefaciens, making the pathogenic strain resistant to the antibiotic. A new product, K1024 was developed as an improvement of K84. The DNA sequence responsible for the ability of the Agrobacterium radiobacter to transfer its immunity to other organisms was identified and deleted.

2. How effectiveness is cow dung and how does it work

It was clarified that cow dung contains other antagonistic microorganisms such as Bacillus subtilis which produce antibiotics that have antimicrobial activity on bacteria.

3. How do oil based products work on *Agrobacterium*—
   Oils in general work by suffocation.
4. Comment: There is need to integrate national policies with information with the industry and ensure that such important information is safeguarded to prevent potential detrimental effects to the industry.

Way forward

1. Crown gall challenge was found to be common in the East African region. The impact of *Agrobacterium* on production and effect on productivity should be established. This should be done in conjunction with the private sector, who own the farms.
2. Management and nursery hygiene may help to control spread.
3. Management and control should be included in the harmonisation efforts in the E. African community particularly through instituting phytosanitary measures and testing of potential pesticides.
4. Proposals should be prepared for management of crown gall, which will benefit the East African region.
5. Research on new products eg *A. radiobacter* K84 should be initiated while waiting for the K1026 to go through the regulatory process.
6. A technical committee should be established to develop trial protocols, testing kits, initiate regional implementation and institute regional quarantine to prevent spread.
7. Collaborative approach to research and regulation to disease management was encouraged. Researchers were encouraged to be proactive and submit proposals that would have an impact on the management of the disease in the region.

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