Pest risk assessment for regulatory control of *Bactrocera invadens* (Diptera: Tephritidae) in the Musina area (Limpopo province).

JH Venter

21933545

Dissertation submitted in partial fulfilment of the requirements for the degree Master in Environmental Sciences at the North-West University (Potchefstroom campus)

Supervisor: Professor H. van Hamburg
Co-supervisor: Professor J. van den Berg

October 2012

ABSTRACT

Fruit flies (Tephritidae) can enter and establish in new territories due to the movement of fruit from one area to another through trade or tourism, which can negatively impact on fruit production and market access. An invader fruit fly species (*Bactrocera invadens*) has established on the African continent and has spread throughout sub-Saharan Africa. This newly described polyphagous fruit fly species is a successful invader species which continues to distribute and establish in new habitats. The introduction and establishment of *B. invadens* in South Africa may have serious market access consequences with regard to fruit exports due to its absence in the territories of many trading partners. The Musina area was considered as the study area as it is the first entry point from Zimbabwe. The national highway (N1) which runs through the area is a major route from several *B. invadens* infested countries in the Southern Africa region. A species initiated pest risk assessment was conveyed to determine the risk potential of this pest. The pest risk assessment (PRA) identified several pathways with a high risk to the Musina area, that *B. invadens* can follow. A detection survey was carried out to determine the status of *B. invadens* in the Musina area as support to the PRA. The detection survey continued over three years and by the second year *B. invadens* was detected for the first time in the study area. The detection survey was followed by a delimiting survey and the pest was eradicated in the area. After several months of no detection, it was however detected again in the area. Risk management options were suggested for regulatory control as an outcome of the pest risk assessment. These measures can be utilised by the National Plant Protection Organisation of South Africa for the commercial importation of host material of *B. invadens*, control of fruit imported by travellers, informal traders and national control in the event of pest incursions in the area. Corrective actions as well as quarantine actions should be implemented in an integrated approach in the affected areas.

Key words: *Bactrocera invadens,* pest risk assessment, detection survey, delimiting survey, eradication, risk management options, integrated approach.

UITTREKSEL

Vrugtevlieë (Tephritidae) kan nuwe gebiede binnekom en vestig deurdat vrugte van een gebied na ‘n ander vervoer word met handel en toerisme, wat ‘n negatiewe impak mag hê op die produksie van vrugte en marktoegang. ‘n Indringer vrugtevliegspesie (*Bactrocera invadens*) het gevestig geraak op die Afrika kontinent en het dwars oor sub-Sahara Afrika versprei. Hierdie nuut beskryfde polifage vrugtevliegspesie is ‘n suksesvolle indringerspesie wat steeds versprei en in nuwe habitatte vestig. Die binnekoms en vestiging van *B. invadens* in Suid Afrika mag ernstige marktoegangs implikasies hê vir die uitvoer van vrugte aangesien dit afwesig is in gebiede van baie handelsvennote. Die Musina area word as die studiegebied beskou aangesien dit die eerste punt van binnekoms vanaf Zimbabwe is. Die nasionale hoofweg (N1) wat deur die gebied gaan is die vernaamste roete vanaf verskeie *B. invadens* geïnfesteerde lande in Suidelike Afrika. ‘n Pes-geïnisieerde risikoberaming is uitgevoer om die risiko potensiaal van die pes te bepaal. Die pesrisiko beraming het verskeie hoë risiko bane vir die binnekoms van *B. invadens* vir die Musina area geïdentifiseer. ‘n Opsporingsopname om die status van *B. invadens* in die Musina area te bepaal is gedoen as ondersteuning vir die pesrisiko beraming. Die opsporingsopname het vir drie jaar voort geduur en eers teen die tweede jaar is die aanwesigheid van *B. invadens* aangeteken in die studie gebied. Die opsporingsopname is gevolg deur ‘n afbakeningsopname en die pes is vervolgens uitgewis in die gebied. Verskeie maande het verloop waartydens geen opsporings in die gebied aangeteken is nie. Opsies vir risikobestuur is geformuleer as regulerende fitosanitêre beheermaatreëls as ‘n resultaat van die pesrisiko beraming. Hierdie maatreëls kan deur die Nasionale Plant Beskermings Organisasie van Suid Afrika gebruik word vir die kommersiële invoer van gasheermateriaal van *B. invadens*, die beheer van vrugte invoere deur reisigers, informele handelaars en vir die nasionale beheer van pes instromings. Herstellende en kwarantyn aksies moet geïmplimenteer word as ‘n geïntegreerde benadering in geaffekteerde gebiede.

Sleutel woorde: *Bactrocera invadens*, pesrisiko beraming, opsporingsopname, afbakeningsopname, uitwissing, risikobestuurs opsies, geïntegreerde benadering

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the many people who provided me with assistance in this research project.

Prof. Huib van Hamburg my mentor and supervisor for his leadership, encouragement and friendship during the study. Prof. Johnnie van den Berg my co-supervisor for his leadership providing accurate and detailed advice. Thank you for your patience in assisting me throughout the period. The support personnel from the School of Environmental Management of the North West University for your professionalism.

The Department of Agriculture Forestry and Fisheries who funded my studies and provided logistical support and resources. With special thanks to Mr. M A Holzhausen who provided support during the study and was always available for advice as well as Ms A P Baxter, the Director Plant Health who made the study possible and provided support throughout. The Plant Health Early Warnings Division and the personnel of the Directorate Inspection Services for their support.

Members of the various fruit industry associations and the farmers in the Musina area.

I would also like to thank my wife Lorette who motivated and encouraged me through the late nights and early mornings and prayed for my safety. My children Melissa and Heinrich who never complained when I worked over weekends and late nights. My father and mother, who taught me perseverance.

Finally, to my Heavenly Father who as always provided me with my needs and strength at the right time.

TABLE OF CONTENTS

TABLE OF FIGURES:

LIST OF TABLES:

LIST OF ACRONYMS:

AFFI: African Fruit Fly Initiative

BAT: Bait application technique

CGA: Southern African Citrus Growers Association

CIRAD: Centre de coopération internationale en recherche agronomique pour le développement

CNEARC: Centre national d'études agronomiques des régions chaudes

CRI: Citrus Research International

DAFF: Department of Agriculture Forestry and Fisheries of South Africa

DIS: Directorate Inspection Services

DPH: Directorate Plant Health

EAC: East African Community

EAPIC: East African Phytosanitary Information Committee

FAO: Food and Agriculture Organization

HPC: Horticulture Promotion Council

IAEA: International Atomic Energy Association

ICIPE: International Centre of Insect Physiology and Ecology

IITA: International Institute of Tropical Agriculture

IPPC: International Plant Protection Convention

ISPM: International Standard for Phytosanitary Measures

MAT: Male annihilation technique

NPPO: National Plant Protection Organization

NPPOZA: National Plant Protection Organisation of South Africa

PFA: Pest Free Areas

PRPV: Programme Régional de Protection des Végétaux

RID: Reseau des Ingenieurs pour le Developpement

RMCA: Royal Museum for Central Africa

RSA: Republic of South Africa

SADC: Southern African Development Community

SIT: Sterile insect technique

SPS: Sanitary and Phytosanitary

UK: United Kingdom

USA: United States of America

USAID: United States Agency for International Development

USDA-APHIS: United States Department of Agriculture - Animal and Plant Health Inspection Service

WTO: World Trade Organization

CHAPTER 1: INTRODUCTION

1.1 Introduction and literature review

Fruit flies (Diptera: Tephritidae) are economically important pests for the fruit industry (White & Elson-Harris, 1994). Many fruit fly species are polyphagous and cause serious damage to a number of crops of different plant families (White & Elson-Harris, 1994; Clarke *et al*., 2005). Not alone do they cause severe damage to fruit crops if control programs are not established, but the mere presence of certain fruit fly species can cause countries to suffer from strict trade restrictions, the loss of market access and subsequently the loss of valuable fruit export income to producers, traders and governments (Follett & Neven, 2006). The most damaging species belong to the genera *Anastrepha*, *Bactrocera*, *Ceratitis* (Previously *Pterandrus*), *Dacus* and *Rhagoletis* (White & Elson-Harris, 1994). Fruit flies of economic importance in South Africa are *Ceratitis capitata* (Wiedemann), *C. rosa* Karcsh, *C. cosyra* (Walker) to several fruit and vegetable crops and *Dacus bivittatus* (Bigot) and *D. ciliatus* (Loew) to cucurbits (Annecke & Moran, 1982).

Most tephritid species oviposit in clusters just below the exocarp of a host fruit which will leave a small puncture mark. The eggs develop into first instar larvae which feed on the pulp of the fruit and migrate deeper into the fruit. Two more instars follow and the third instar emerges from the fruit to fall on the soil or litter surface (Peña *et al.,* 1998). Fruit fly larvae pupate after burrowing into the soil from where adult fruit flies emerge. Fruit flies are generally strong flyers, disperse quickly and may live for several months (White & Elson-Harris, 1994).

The oviposition marks are small and can easily be overlooked by plant quarantine inspectors during inspections. However, if a fruit with oviposition marks is detected it can be rejected based on quality as well as phytosanitary reasons. Large quantities of fruit may also be rejected on puncture marks alone without any verification process in place to determine if there are viable eggs or larvae in the fruit (Stonehouse *et al*., 2004).

The second and third instar feeding damage and subsequent secondary microbial rotting that follows, renders fruit inedible to humans (Peña *et al.,* 1998). However, rotting causes the infested fruit to be detected easier and as such fruit is unacceptable for marketing or consumption, it would be rejected. Fruit infested with fruit fly larvae which were undetected at first (egg and first instar larval stage) is often discarded after transport or importation by marketers or consumers (Stonehouse *et al*., 2004). Fruit flies emerging from discarded fruit can establish in new areas and countries outside their native range as long as there are suitable and enough hosts available and the climatic situations are favourable (De Meyer *et al.,* 2008; EPPO, 2010; Vayssières *et al*., 2009a). Fruit flies are transported very successfully through either commercial fruit, or as part of traveller's luggage from one country or continent to another (Frampton, 2000).

During a fruit fly survey in Kenya in 2003 an exotic fruit fly closely related to *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) was detected (Lux *et al.,* 2003). Thereafter it was detected in several other African countries such as Tanzania (Mwatawala *et al*., 2004). It was described in 2005 as *Bactrocera invadens* (Drew, Tsuruta & White) (Diptera: Tephritidae) and considered to belong of the *Bactrocera dorsalis* species complex (Drew *et al.,* 2005; White*,* 2006). The species group is included within the subgenus *Bactrocera* and the name can be cited as *Bactrocera* (*Bactrocera*) *invadens*. Genetic studies as well as parapheromone studies identified this species as very close to *Bactrocera dorsalis* although, with certain morphological differences (Khamis *et al.,* 2009; Tan *et al*., 2010; Liu *et al*., 2011). Several of the species within the *B. dorsalis* complex have very close genetic relations such as *B. papaya* Drew & Hancock and *B. carambolae* Drew & Hancock (Muraji & Nakahara, 2002). The origin of this fruit fly may be Asia due to the geographical origin of *B. dorsalis.* Furthermore *B. invadens* has been recorded from Sri Lanka, India and Bhutan (Khamis *et al.,* 2009; EPPO, 2010).

Several species of the genus *Bactrocera* are of high economic importance as they have a high reproductive rate, successful dispersion and a wide range of fruit hosts causing severe damage (Bateman *et al.,* 1978; White & Elson-Harris, 1994). The genus *Bactrocera* is a large genus with in excess of 400 recognised species (White & Elson- Harris, 1994).

B. invadens was detected all across Africa by 2005 (Drew *et al.,* 2005) at different climatic conditions and reported to have a wide host range in Africa. It has been recorded on more than 70 host species from 25 plant families in Africa which includes both cultivated and wild plants (De Meyer *et al.,* 2012; Mwatawala *et al.,* 2006a; Rwomushana *et al.,* 2008a). Most of these records were obtained from adults reared from fruits collected in field surveys as well as from host preference studies under laboratory conditions (Rwomushana *et al.,* 2008a).

It is a recognized pest in many of the countries where it occurs and utilizes economically important fruit cultivars from different plant families, such as *Mangifera* (Anacardiaceae), *Citrus* (Rutaceae) and *Psidium* (Myrtaceae) species as hosts (Rwomushana *et al.,* 2008a;

Vayssières *et al*., 2009a; EPPO, 2010). Based on the existing host plant records, *B. invadens* can be considered to be a polyphagous species (Aluja & Mangan, 2008), however, the full extent of its host range is still not known (Rwomushana *et al.,* 2008a; Mwatawala *et al.,* 2009).

B. invadens was collected regularly not only from mango in east and west Africa, but also from other economically important fruit hosts such as citrus, guavas and bananas (Abanda *et al*., 2008; Vayssières *et al*., 2005; Vayssières *et al*., 2009a ; Mwatawala *et al*., 2009; Goergen *et al.,* 2011).

The pest caused severe damage to crops in Africa especially where no fruit fly management programs are followed and can create food security problems in some regions (Kwasi, 2008; Ekesi *et al*., 2006; Ndiaye *et al*., 2007; Vayssières *et al.,* 2009a). It is found in various habitats and early studies indicated that the pest prefers coastal lowland areas but it has been found in habitats up to 1200m above sea level (Ekesi *et al.,* 2006). In Kenya and Tanzania, mango, banana and several citrus varieties were significantly affected by *B. invadens* (Mwatawala *et al.,* 2006b; Rwomushana *et al.,* 2008a). Although several fruit fly species may be pests on Mango in Africa, up to 76% damage was recorded as result of *B. invades* infestation in Kenya (Ekesi *et al.,* 2006). This pest has a high fecundity and at 28 ± 1ºC the mean generation time is 31 days (Ekesi *et al.,* 2006) which is shorter than that of indigenous fruit flies such as *Ceratitis cosyra* (Walker) and it was reported that *B. invadens* would be able to displace indigenous fruit fly pests (Ekesi *et al.,* 2009).

The cost of controlling fruit flies throughout the crop production cycle may be too high for subsistence, emerging and small scale farmers (Stonehouse *et al*., 1998). Post harvest treatments to disinfest fruit may lead to additional quality losses and is expensive to implement which would make it difficult to maintain or enter export markets (Follett & Neven, 2006; Griffin, 2000).

There is also evidence of a mutually beneficial relationship between certain *Bactrocera* species and orchid species from the genus *Bulbophyllum* as the orchid flowers excrete a synomone which attracts males for pollination (Tan & Nishida, 2000; Tan *et al*., 2006). Control measures should therefore be considered carefully so as not to impact negatively on the environment. Several species of the genus have unique pollination roles in their native range (Tan *et al*., 2002; Tan *et al.*, 2006).

The current spread through Africa and the expansion of the host range is a concern to other countries across the world where the pest does not occur regarding the risk this pest poses when consignments of host plant material is traded (EPPO, 2010). Phytosanitary measures were implemented by a number of countries to prevent entry of the pest to their territories. Until 2011, specific commodity based post harvest treatments had not been published to disinfest host material from *B. invadens*. This was due to the fact that it was only described in 2005 and no such studies had been completed (Manrakhan *et al.,* 2011). However, by 2011 new studies did suggest the effectiveness of post harvest cold treatments especially for citrus fruit (Grout *et al.,* 2011; Hallman *et al.,* 2011). Further studies also developed cold treatment schedules for "Hass" Avocado in 2012 (Ware *et al*., 2012).

The discovery of this fruit fly in Africa as well as the spread throughout equatorial Africa and the southern movement of the pest caused the South African authorities to impose strict trade restrictions of host material from known infested countries and to increase exotic fruit fly surveillance actions as precautionary measures. The introduction of *B. invadens* to South Africa will probably influence market access as well as fruit production. This will have a negative impact on job creation, economic growth and development in the agricultural sector. South Africa and Mauritius suspended the importation of mango and avocado from Kenya until suitable mitigation options can be agreed upon between the trading partners (Ekesi *et al.,* 2009). In 2008, the United States issued a federal import quarantine order for host products of *B. invadens*, with strict import conditions, which was later reviewed in 2009 (EPPO, 2010; USDA-APHIS, 2008). The European Plant Protection Organisation (EPPO) included *B. invadens* in their list of quarantine pests in 2010 (EPPO, 2010). A pest initiated pest risk analysis (PRA) is required to ensure that technically justified risk mitigation measures are implemented to reduce the risk of establishment of this pest in new areas. Recently published research and existing PRAs will greatly assist with such a PRA (EPPO, 2010).

The World Trade Organisation formulated the Agreement on the Application of Sanitary and Phytosanitary Measures (WTO-SPS) to promote trade between member countries with an acceptable pest and or health risk. The WTO-SPS became obligatory to member countries on 1 January 1995. Member countries can implement regulations to protect humans, animals and plants in their territories from the entry of harmful foreign organisms. Members should base these regulatory measures on scientific data. The WTO accepts three standard setting bodies: the OIE (International Office of Epizootics, for animal health), Codex Alimentarius (food safety standards for humans), and the International Plant Protection

Convention (IPPC) (WTO, 1995). The purpose of the IPPC is to prevent the introduction and spread of plant pests, and to promote appropriate control measures (FAO, 2009).
South Africa is a signatory member of the WTO-SPS and the IPPC under the auspice of the Food and Agricultural Organisation (FAO). As a result, South Africa is obligated to establish and maintain a National Plant Protection Organisation (NPPO). The IPPC ensures that International Standards for Phytosanitary Measures (ISPMs) are compiled and published which guide member countries to fulfil their obligations (Quinlan, 2002). Standards and guidelines for pest risk analysis, surveillance, eradication plans and other measures or actions are drafted by the IPPC for member countries to ensure the safeguarding of their territories from the introduction of new plant pests (FAO, 2009).

The NPPO of South Africa (NPPOZA) is seated within the Department of Agriculture Forestry and Fisheries (DAFF) and the actions are managed and executed by a policy directorate and two operational directorates. Regulatory actions are executed by the NPPOZA under the Agricultural Pests Act 1983, (Act No. 36 of 1983) and include the implementation of control measures for pest incursions as far as the Act allows (Anonymous, 1983).

NPPOZA is obligated to follow a scientific process, namely a pest risk analysis (PRA) to develop technically justified import regulations (phytosanitary measures) that will ensure trade between trade partners, which protects the importing country from pests but is not creating unnecessary barriers to trade.

A pest risk analysis (PRA) is 'the process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it' (FAO, 2009). A pest is "any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products" (FAO, 2009). Countries have adopted and used different models to conduct PRAs worldwide. The assessment can be qualitative or quantitative. A PRA can be pathway initiated or pest initiated (FAO, 2009). The pest risk assessment procedure is used to determine the most likely pathways of introduction, places of introduction, areas of establishment and areas of highest economic or environmental impact within a country or regulated area for a specific pest (Baker & Macleod, 2005; FAO, 2009). Factors such as the climatic threshold of a particular pest, the rate of production and host plants in an area are evaluated.

Pest risk management is the "evaluation and selection of options to reduce the risk of introduction and spread of a pest" (FAO, 2009). This stage would follow the pest risk assessment stage after the outcomes have been documented. When the aim is to draft import regulations for host plants of a specific pest, the PRA should be conducted according to international standards which are least restrictive to trade (De Hoop, 2005). Phytosanitary measures are implemented for the control of a pest within a country. The aim of such phytosanitary measures is to eradicate or to contain a pest in a specific area and must be technically justified (Quinlan, 2002; De Hoop, 2005).

According to Article IV, *The Revised Text* (1997) (FOA, 2009) of the IPPC, the NPPOZA is also responsible to initiate and carry out surveillances of cultivated and wild plants. This will determine the status (presence) of pests in the country with the objective to report the occurrence, outbreak and spread of such pests and the control of it (Quinlan, 2002). Article VIII describes the importance of the obligation to notify pest occurrences and the status of such pests to trading partners (FAO, 2009).

Pest surveillance forms an important component to verify the outcomes of a PRA although it is not regarded as a separate stage of the PRA process. A change in status of a quarantine pest in a territory should cause amendments to the import regulations or phytosanitary measures implemented by the NPPO after notifying international trade partners. Notification of changes in the status of occurrence of the pest can be done through official notification systems made available by the IPPC and the WTO. Changes of pest occurrence in an area may result in a relaxation of phytosanitary measures for the importation of produce, or alternatively it may implement stricter phytosanitary measures for export commodities which are destined for countries or areas free from the pest (FAO, 2009). It is therefore just as important for a country to know where pests are occurring in its territory as it is to know where they do not occur.

The introduction and spread of exotic fruit fly species such as *B. invadens* on the African continent emphasized the need for the NPPOZA to develop a surveillance plan for exotic fruit fly pests. The surveillance program also formed an early warning system for the NPPOZA to allow for a reasonable chance to react and control the spread of the pest to other areas in the country after early detection (Barnes & Venter, 2008).

Several International Standards for Phytosanitary Measures have been approved which describes surveillance for the application for different purposes and for fruit flies. ISPM No. 6, *Guidelines for surveillance* (1997), describes the general components of survey and monitoring systems for the purpose of pest detection (Quinlan, 2002). ISPM No. 6, forms a basic standard which also supports the formulation of other standards as it provides guidelines to supply information regarding pest occurrence which can be used in pest risk analyses, the establishment of pest free areas, pest free places and sites of production, areas of low pest prevalence and where appropriate, the preparation of pest lists. ISPM No. 26 Establishment *of Guidelines for Pest Free Areas* (Tephritidae) (2006) (FAO, 2009) describes the specific guidelines for different types of surveillance objectives to establish and maintain pest free areas for fruit flies. Trap types and lure types with trapping densities for fruit fly species are described as an appendix to this standard (FAO, 2009).

Fruit fly trapping protocols are developed theoretically but may not be practical as it is dependent on availability of hosts, accessibility of the terrain and agricultural activities as well as the social impact (USDA-APHIS, 2006) and need to be amended once trap placement commences. Trapping procedures of fruit fly species will predominantly consist of a male attractant program and a food bait program targeting females. Male fruit flies are often also attracted to protein baits. Some species such as *Anastrepha* sp. do not respond to male parapheromone lures, but are responsive to several protein based bait combinations to a greater or lesser extent (White & Elson-Harris; 1994, IAEA, 2003; Thomas *et al*., 2001).

Higher frequency trapping with closer grid intersect points covering larger areas, increases the probability of point incursion detections during detection surveys with exclusion as an objective as well as early detection and response (Clift & Meats, 2005). However, one fruit fly specimen detected in a surveillance trap is not representing the total population number, since the trap frequency and the distance apart only provides an indication of the number of fruit flies in an area (Meats, 1998). One discarded infested fruit may have several adult fruit flies emerging from the soil at the point where the fruit was discarded and they may disperse in several directions (Clift & Meats, 2005). Traps can be made more efficient by rotating the trapping locations according to the ripening of host plants, or by simply increasing the total number of traps (IAEA, 2003). A fruit cutting or rearing survey can also be carried out to support evidence regarding the occurrence status of a pest in a particular area and to determine which hosts are infested (Kwasi, 2008; IAEA, 2003).

The NPPOZA initiated an exotic fruit fly surveillance program in 2006 to target mainly exotic *Bactrocera* species (Barnes & Venter, 2008). This surveillance included the placement of parapheromone baited traps at port of entries, production areas, urban areas and road transects (Barnes & Venter, 2008). Surveillance is conducted countrywide and coordinated from a central point in the NPPOZA. The NPPOZA utilises expertise within itself as well as within the major fruit industries such as the citrus, subtropical and deciduous fruit industries. The detection survey aimed to detect, as a first priority, the potentially invasive fruit fly pests such as *Bactrocera cucurbitae*, *B. invadens, B. latifrons* and *B. zonata* (Barnes & Venter, 2008).

The traps set out in the survey consisted of bucket type traps such as the Chempack bucket trap, and the Moroccan trap (Barnes & Venter, 2008; FAO, 2009; IAEA, 2003). These traps were baited with male specific pheromones or with protein hydrolysate bait (Barnes & Venter, 2008).

After *B. invadens* established in Africa it started to spread to new countries. From 2003 until 2008 it has spread to northern Mozambique (Correia *et al.,* 2008), northern Namibia and Zambia (De Meyer *et al.,* 2012). This tendency indicated that there was a southern movement of the pest and the northern Limpopo area was identified as a possible risk area for incursions.

Surveillance programs for the detection of exotic fruit flies have been developed by the NPPOZA since 2006 for the protection of the export fruit industries of South Africa as well as the development of the small scale farmer (Barnes & Venter, 2008). The detection survey developed by the NPPOZA served as an early warning system for exotic fruit flies (Phelong, 2005). The detection of a single exotic fruit fly will activate a delimiting survey to determine the extent of the spread (Barnes & Venter 2008). The NPPOZA developed an action plan during 2008 specifically for the detection and rapid response in reaction to the detection of *B. invadens* (Manrakhan *et al*., 2009).

A delimiting survey was initiated after the detection of *B. invadens* in the surveillance area in 2010 which led to the area to be placed under quarantine by the NPPOZA (Manrakhan *et al.,* 2011). The quarantine covered an area of approximately 1200 km^2. Control measures were implemented and the pest was eradicated from the area (Manrakhan *et al.,* 2011). The NPPOZA also triggered a fruit sampling survey after the positive identification according to Drew *et al*., (2005) of *B. invadens* in the area. Samples of fruit were collected at all production sites which were placed under quarantine (Manrakhan *et al.,* 2011).

For the purpose of this study, a pest risk assessment for the Musina area was carried out which identified specific risk areas with regard to pathway, entry, establishment and spread. Management options were further proposed as a result of the risk assessment, taking into consideration the results of the detection survey and existing control measures. These management options can be applied for the rest of South Africa, taking into consideration the risk factors identified for the Musina area. This is particularly important as the number of detected specimens in the area were extremely low which contributed to the success of the eradication program.

1.2 Aim and objectives of this study

The aim of this study was to develop the methodology to determine the risk of *B. invadens* establishing in the Musina area to fruit production in the Limpopo province and to the rest of South Africa.

Specific objectives were:

- to develop a science based species initiated pest risk assessment for *Bactrocera invadens*
- to develop a protocol for and initiate a detection survey using male specific parapheromones and protein baited traps and to follow up with a delimiting survey after detection
- to assess current control options for the importation of host plants and plant products in affected areas and to recommend options for control based on the above outcomes.

CHAPTER 2: MATERIALS AND METHODS

2.1 Qualitative species initiated pest risk analysis of *Bactrocera invadens* (Diptera: Tephritidae).

This PRA has been carried out in accordance with the International Standards for Phytosanitary Measures (ISPM) of the IPPC. The PRA consisted out of three stages (FAO, 2009) namely an initiation, assessment, and pest management stage, as illustrated in Figure 2.1. However, the pest risk assessment was supported by a detection survey to determine the presence of this pest in the study area due to the unknown status of *B. invadens* in the study area when the study was initiated. The initiation and assessment stages are discussed in Chapter 3 together with the results of the detection survey. The results of stage one and two of the PRA were evaluated together with the outcomes of the detection survey and subsequent delimiting survey to suggest a pest management strategy. Management options or the pest risk management stage were therefore discussed separately in chapter 4.

The pest risk assessment stage included pest categorization, assessment of the probability of introduction and spread, and an assessment of potential economic consequences with respective interrelations between these three steps (FAO, 2009). In this stage relevant technical information about the pest's biology, hosts, distribution, tolerances as well as the climatic conditions in areas where it currently occurs and for those areas where it occurs in the importing country or area were studied (Fig. 2.1).

The pest risk management staged followed the assessment stage. However, for the purposes of this study the outcomes of the survey conducted in the PRA area were also considered to establish a higher degree of certainty regarding the occurrence of the pest in the area and preceeded the management stage (Fig.2.1).

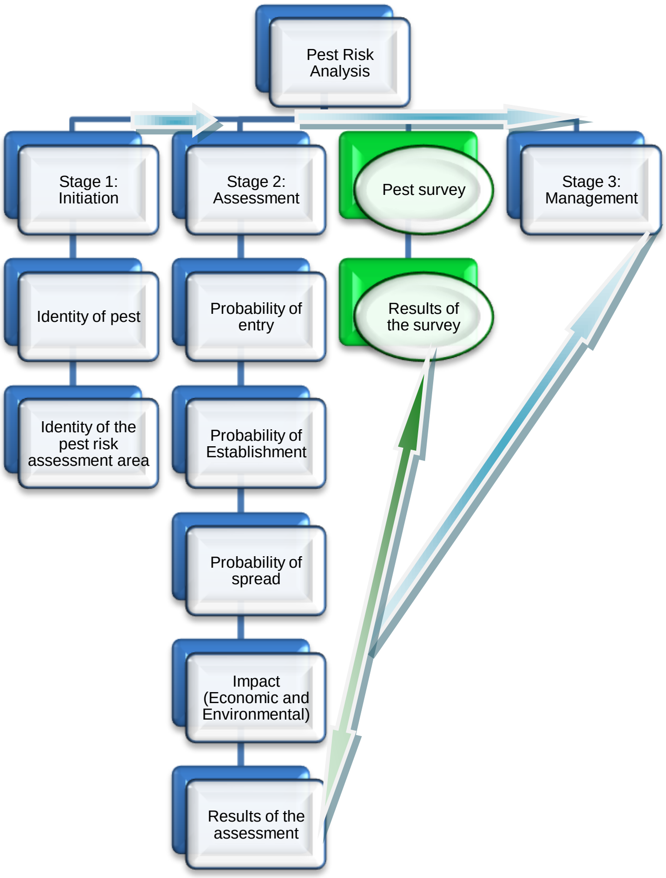

Figure 2.1: Different stages of the pest risk analysis indicating the adaptation made to include a pest survey. Results of the pest risk assessment and the pest survey were considered to suggest pest management options.

Technical information (about the pest's biology, distribution and damage it causes) was studied in stage two of the PRA to justify the application of phytosanitary measures which were proposed in the third stage of the PRA, the management stage. The measures proposed can be used to prevent the introduction of the pest into a regulatory area by regulators such as the NPPOZA (FAO, 2009).

The results of the assessment stage of the PRA are based on a literature review and are supported by the results of a qualitative survey. All possible hosts and pathways were investigated in terms of the point of entry and distribution points (Aluja & Mangan, 2008).

Pest risk for plant hosts consists of two major components: a) the probability of a pest entering, establishing and spreading in the study area and b) the consequences or impact it may have should this happen. These two components were combined to provide an overall estimate of the risk. Biological information was obtained from all areas where the pest currently occurs which provides evidence of the ability of a pest to be associated with a pathway and to survive in transport or storage (FAO, 2009).

Spread potential was determined by studying available information of the distribution of hosts in the area as well as the availability of wild host plants and ornamental garden plants (FAO, 2009).

Entry by natural means was also assessed. While there is usually no control over this aspect, hosts in the natural area could provide evidence on the possible rate of spread. The pest must be able to disperse from the entry commodity or pathway to a mating partner as well as a new suitable host to enable it to multiply and to start a new population (Baker & Macleod, 2005). Important factors considered were climatic thresholds and reproduction rate, specifically the time it takes for a known population to double. If limiting factors are known such as altitude and temperature, thresholds climatic modelling can be done from data available in areas where the pest already occurs (Baker & Macleod, 2005). For this study, published establishment estimates, with available existing climatic data were used to determine the probability of establishment (De Meyer *et al*., 2010; EPPO, 2010). The availability, quantity and distribution of hosts in the PRA area affect the risk of establishment (FAO, 2009). The population size that is required for establishment in relation to the type of hosts is important (Baker & Macleod, 2005). However, as for many new pests, this information was also not available for *B. invadens* when the PRA was conducted.

The PRA also examined the consequences of the pest on social and economic factors in terms of production losses and loss in export markets (Baker & Macleod, 2005). The direct and indirect economic consequences (FAO, 2009) on production and trade of different fruit hosts in the Musina area were assessed as well as the effect this pest may have on market access and the maintenance of export markets.

The purpose of this pest risk assessment was to review and prioritize existing information, based on the identity of the pest, the proximity to the area, the regulatory status and establishment and spread potential as well as economic importance (Baker & Macleod, 2005). Assessments of the possible and probable pathways with which a pest may enter were followed (FAO, 2009).

2.1.1 Initiation (Stage 1).

The initiation phase documented the reason(s) for initiating this species-initiated PRA. Initiation would usually be triggered after a new pest that was detected in the country, or such a pest was intercepted from imported host material, or as an import request of the pest, or as a result of a pest that is spreading rapidly and may enter the country and the revision of existing phytosanitary regulations and control options were investigated. After the pest was identified as *B. invadens* the PRA area was identified and described as it was realised that phytosanitary measures would be required for the importation of host plants and plant products of this pest into the PRA area. A brief background of the pest was provided and the impact it had in Africa since it was first detected in 2003.

2.1.2 Qualitative species initiated risk assessment (Stage 2).

The assessment was preceded by a study of the pest's biology, geographic occurrence and behaviour in the countries it already occurs. Previous risk assessments for the same pest were also taken into consideration. The European Plant Protection Organisation (EPPO) has drafted a species initiated qualitative PRA for *B. invadens* for the introduction of host material to the EU member countries, from countries where the invader fruit fly occurs (EPPO, 2010).

For the purposes of this study a species initiated Pest Risk Analysis conducted by the EPPO for *B. invadens* was utilised as a reference source with adaptation to the risk assessment model used by EPPO regarding the South African circumstances and specific risks for the Musina area as well as for the country.

The general principles and guidelines in terms of the International Standards of Phytosanitary Matters (ISPM) of the IPPC were followed (FAO, 2009). Three ISPMs were used as guidelines namely 1) ISPM No. 2 (Framework for pest risk analysis, 2007), 2) ISPM No. 11 (Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms, 2004) and 3) ISPM No. 21 (Pest risk analysis for regulated non-quarantine pests) (FAO, 2009).

Information about the study area was gathered through publications and general statistical data such as climatic conditions, and trade of potential host plants. Visits to the area, interviews with producers, observations of host plants and general trade as well as movement of potential host material into and through the area further supported the risk assessment. The qualitative species initiated PRA was compiled after evaluating available information about the pest and the hosts produced in the PRA area. This PRA considered the level of risk to be a product of probability and consequences.

2.1.2.1 Probability of entry

The assessment of the probability of entry was divided into two, namely, the probability of importation, or natural spread and probability of distribution from an entry point. The probability of entry described the probability that *B. invadens* will enter through importation or natural spread into the Musina area, which was assessed in terms of the different pathways identified and its ability to survive processes and conditions involved with the pathway. The possible pathways the pest can follow was identified and described.

The probability that the pest will disperse and distribute was assessed, because of the movement of host material in the PRA area after importation. The movement of host material such as fresh fruit can include fresh fruit destined for processing, sale or disposal of fruit to the local community which may lead to the transfer of the pest to a susceptible new host.

The factors identified to be considered in the assessment of the probability of entry were:

- ❖ Geographical distribution
- ❖ Availability of host plant commodities
- ❖ Incidence of the pest likely to be associated with identified pathways
- ❖ The probability of importation and the survival potential of the pest following the pathway
- ❖ Probability of dispersal, including mechanisms and probability of the pest, to allow movement from the pathway to a suitable host
- ❖ Whether the imported commodity is to be sent to a few or many destination points in the PRA area
- ❖ Proximity of entry, transit and destination points to suitable hosts
- ❖ Risks from by-products and waste

2.1.2.2 Probability of establishment

Information about the pest such as life cycle, host range, climatic conditions and natural biomes where the pest currently occurs was obtained from areas where the pest currently occurs to estimate the probability of establishment.

This was compared with the host plants cultivated and occurring naturally in the PRA area and with the climatic conditions and biomes in the area.

These factors taken into account included:

- ❖ Availability of suitable hosts in the PRA area and land use data
- ❖ Pest control measures applied in the area

- ❖ Reproductive strategy and survival of the pest
- ❖ Suitability of the environment for the pest to establish according to existing published climatic models for the pest and prevailing rainfall and temperature in the PRA area

2.1.2.3 Probability of spread

Spread is defined by the IPPC as 'the expansion of the geographical distribution of a pest within an area' (FAO, 2009). It was considered to occur after establishment, thus the probability of spread considered factors relevant to the movement of the pest, after establishment in the PRA area, to other susceptible host plants of the same or different species in other areas. The biological information obtained from areas where the pest currently occurs was used to compare with the situation in the PRA area and the rest of South Africa.

Factors influencing the probability of spread included:

- ❖ Suitability of the natural and/or managed environment for the natural spread of the pest
- ❖ Presence of natural barriers
- ❖ The potential for movement with commodities, conveyances.

The risk related probabilities to determine entry, establishment and spread potential were rated by assigning it a high, medium, low or negligible risk estimation rating.

2.1.2.4 Economic consequences

The consequences that *B. invadens* may have on the economy were assessed by addressing direct and indirect economic impacts or consequences. The severity or magnitude of the pest's consequences was determined by providing a high, medium, low or negligible rating. Direct pest effects took into consideration the effects the pest may have on plant life or health (loss of production).

Indirect pest effects included the consequences the pest may have on domestic as well as international trade in terms of loss of market access or additional measures implemented to enable trade.

The factors which were taken into consideration to assess the economic consequences were:

- ❖ Production of host types in the PRA area;
- ❖ Consequences as a result of the loss of export markets;

❖ Consequences as a result of production losses and cost of production;

❖ Consequences as a result of environmental impact.

2.1.2.5 Overall assessment

The overall pest risk was determined by combining the probability of an event occurring (entry, establishment and spread) and the consequences if or when it may occur (economic and environmental consequences).

Overall pest risk was determined by taking into consideration the following:
Pest Risk = (probability of introduction) x (magnitude of consequences)
When **probability of introduction** = (probability of entry) x (probability of establishment) x (probability of spread)

And when **magnitude of consequences** = (economic consequences) + (environmental impacts).

The ratings for probability and consequences were placed in a matrix to assist with the decision making process. Information from existing PRAs were included in this risk assessment as indicated in Fig. 2.2

Probability of pest entry establishment and spread				
High	Negligible	Low	Medium	**High**
Medium	Negligible	Low	Medium	**High**
Low	Negligible	Low	Low	Medium
Negligible	Negligible	Negligible	Negligible	Negligible
	Negligible	Low	Medium	**High**

Consequences of pest entry establishment and spread

Figure 2.2: Risk Estimation Matrix for *B. invadens* indicating probability of entry, establishment and spread multiplied by the consequences of entry, establishment and spread. The vertical and horizontal arrows indicate towards an increase of probability and consequences respectively.

2.1.3 Pest Risk Management

The NPPOZA already has preventative import requirements and control measures in place with regard to the importation of host plants products of *B. invadens*. However, these measures were initially put in place as a result of a precautionary approach after *B. invadens* was first reported in Africa, and when very little information was available about this pest. These measures were evaluated against the outcomes of this pest risk assessment and the outcomes of the detection survey of *B. invadens* in the PRA area for each of the pathways identified and documented in Chapter 4. The current role player engagements and interactive forums which the NPPOZA initiated for the prevention and control of *B. invadens* were also evaluated. New measures for the control of *B. invadens* were suggested regarding each of the pathways identified taking in to consideration that through the standard setting process of the IPPC generic management procedures are developed (Follett & Neven, 2006). The overall pest risk rating was used as the level of risk this pest poses to the PRA area and the measures suggested were aimed to lower that risk to an acceptable level of protection (Vayssières *et al.,* 2009b).

Taking the overall risk into account, management measures were drafted in terms of the risk ratings of the different pathways.

Principles for pest risk management were used according to ISPM No. 11 so that the risk can be managed to obtain the required degree of protection in a justifiable and feasible way. Phytosanitary measures identified were evaluated for efficacy, feasibility and consequences (FAO, 2009).
The measures developed were therefore a combination of existing measures and new measures. Mitigation measures were identified to lower the risk of the pest to an acceptable level (Follet & Neven, 2006). Corrective actions and quarantine measures and actions (USDA-APHIS, 2006) were developed in the case of new incursions to isolate a potentially infested area from the rest of South Africa (Manrakhan *et al.,* 2009). This is essential to prevent the spread of the pest and to ensure uninterrupted trade as best as possible (FAO, 2009). Control measures were suggested to contain and eradicate *B. invadens* after it had been detected in a specific area in South Africa. These suggested measures aimed to assist the application of the existing control measures of R110 of the Agricultural Pests Act, 1983 (Act No. 36 of 1983) and the action plan (Manrakhan *et al.,* 2009).

2.2 Specific survey for *Bactrocera invadens.*

2.2.1 Materials and methods.

The detection survey used the existing ISPMs as indicated in Chapter one as well as survey plans used by the NPPOZA as reference for the survey conducted in the study area. A network of traps baited with food baits or pheromones to lure the fruit flies into the trap was set up (Phelong, 2003).

To determine the status of *B. invadens* in the study area as well as to further supplement and substantiate the pest risk assessment, a detection survey was developed for the Musina area which incorporated the objectives of the national exotic fruit fly surveillance program of the NPPOZA and the *B. invadens* action plan. The survey described in this study started in 2009 and had the objective to intensify surveillance in the northern Limpopo areas, especially after the official notification of the presence of the pest in the northern Mozambique and Namibia as well as in Zambia during 2008 by the respective governments. No surveillance records or actions were available at that stage from Botswana or Zimbabwe which increased the probability that the pest could have extended its range unnoticed. Fruit fly traps at host production areas were placed close to national roads since the risk is higher that fruit flies can enter by following the pathway of fruit carried by passengers or truck drivers (Clift & Meats, 2005). Fruit fly traps were placed next to the Limpopo River that forms the border between South Africa and Botswana and Zimbabwe and the service road that follows the border fence, as well as the R572 and the N1.

A grid interception method was used to place traps in the urban area of Musina (Barnes & Venter, 2008). A theoretical grid with trap placement points at grid intersects was placed over a map of the town or area (Vargas *et al.,* 2010). The trap placement points acted as trapping sites where traps baited with different lures were placed close to each other (Meats, 2008). Care was taken not to place these traps too close to each other as they can negatively affect the attractiveness of the lure (Frampton, 2000; Meats, 1998). The availability of fruit in production areas influenced trap placement, however permanent surveillance traps remained in the fruit production areas. This optimised the trapping network for the area over a specific period (Clift & Meats, 2005).

Host plant density, the type of host plants in the area as well as the biology of the fruit fly in terms of the period it takes to complete its life cycle, and dispersal rate was taken in consideration to determine the trap lay out (IAEA, 2003). Host fruit phenology is important to determine the times of the year when fruit is ripening in the area (Vargas *et al.,* 2010). Citrus and tomato fruit were the dominant host cops produced in the area and the concentration of traps around these production sites were increased.

For the purpose of this detection survey, surveillance was done in the Musina area adjacent to the Limpopo River. The surveillance area covered an area from the Pontdrift border post in the east, adjacent to the Botswana border, to the Beitbridge area in the west adjacent to the Zimbabwe border. The Limpopo River forms the northern and western border with the R572 and N1 roads forming the southern and eastern borders respectively (Fig. 2.3).

During the development and planning of the fruit fly survey different role players were approached for cooperation as it was carried out on an area wide level. These included different spheres of government, fruit industry members such as Citrus Growers Association, Citrus Research International, ZZ2 farms, Musina Pack, Tiger Brands Limited and local land users and producers of host plants. Community and industry involvement helped to avoid uncertainties or resistance to the program and enhanced cooperation. It also increased awareness regarding the general pest importance in the area and information exchange in respect of host plants produced in the area. A trapping procedure was drafted to ensure all role players follow the same methodology (USDA-APHIS, 2006). Information described in the procedure included types of trap used, lure types, killing strips used and placement servicing and data management (USDA-APHIS, 2006; IAEA, 2003). The trap densities differed between different areas; a point of entry had a higher density for detection than a production area (IAEA, 2003).

Often fruit fly surveillance is considered to be a temporary or sporadic event but in this detection survey it formed part of an exclusion program and the surveillance traps in the area were placed on a permanent basis, each with a permanent location and unique trap number. A long term budget had to be drafted to ensure enough funds were available and a cost benefit analysis was considered by the NPPOZA but not for this study. However, the costs of this study were carried by the NPPOZA.

A major challenge with a detection survey is to develop a trapping density that is serviceable with the available capacity. Traps should be visited as frequently as possible, preferably once per week (USDA-APHIS, 2006). However, for this study a schedule to service the traps was worked out in order to visit traps consistently in all the areas at a rate of once per month (IAEA, 2003). A delimiting survey for fruit flies can be described as an intensified trapping grid which aims to establish the boundaries of an area considered to be infested by or free from a pest. An area of 25 to 32km^2 surrounding the trap position with the first positive exotic fruit fly detection was delimited (FAO, 2009; Manrakhan *et al.*, 2009).

The detection survey in the area was suspended after the first detection of targeted fruit fly species and a delimiting survey was implemented. The delimiting survey was terminated after a regulatory or management decision was made by the NPPOZA regarding the outcome of the delimiting survey. After termination of the delimiting survey the detection survey was reinstated as the targeted fruit fly species had been eradicated from the area. Thereafter the area was again declared as a pest-free area (FAO, 2009).

The surveillance area in terms of the physical entities and agricultural activities of a particular area remained the same. The servicing frequency was increased to a minimum of one week intervals.

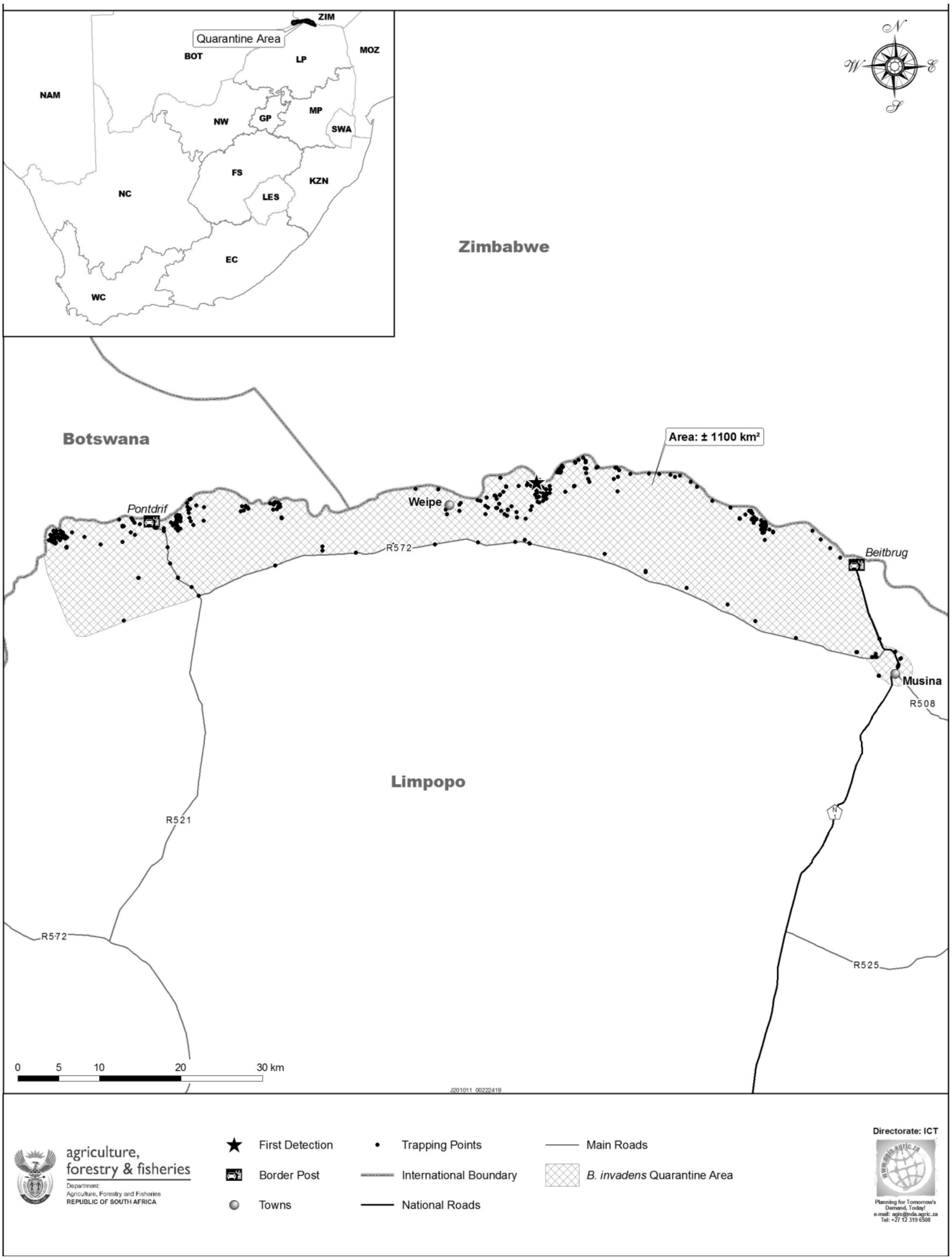

Figure 2.3: Study area indicating the surveillance points for the detection, delimiting and eradication of *B. invadens* (Manrakhan *et al*., 2011).

2.2.1.1 Trap type

Two bucket trap types were used during the survey, Chempack bucket traps and Moroccan traps. The Chempack bucket trap (Chempack, South Africa) is a Mc Phail type trap suitable for dry or wet lures. It has a funnel shaped bottom entrance with three side holes. The lid is translucent and has a basket which contains the killing agent. The bucket is bright yellow (Fig. 2.4) and suitable lures can be placed in the bottom. The Moroccan trap (Insect Science, South Africa) is a bucket with four entrance holes in the side with a basket for the killing strip in the lid (FAO, 2011). It is suitable for dry lure application. It is mostly white in colour (Fig. 2.5) but can also be translucent.

Figure 2.4: Chempack bucket trap used for the detection of *Bactrocera invadens* with two of the three sides and the bottom entrance holes visible.

Figure 2.5: Moroccan bucket trap used for the detection of *Bactrocera invadens* with one of the four side entrance holes visible.

2.2.2.2 Attractants

Two attractants were used for *B. invadens,* namely methyl eugenol polymer plugs (Chempack, South Africa) and Biolure 3 component lure (Chempack, South Africa). Methyl eugenol is a powerful pharapheromone used to attract males of several species from the Genus *Bactrocera* (White & Elson-Harris, 1994). The attractant is used for species within the species complex of *Bactrocera dorsalis*. In the appendix to ISPM No. 26 the IPPC recommends the usage of methyl eugenol lures as it has been used successfully to detect *B. invadens* in several African countries (Drew *et al*., 2005; Rwomushana *et al*., 2008a; FAO, 2011). Biolure 3-component lure was used as a protein lure which consists of different protein hydrolysate components namely, ammonium acetate +, putrescine + trimethylamine (Manrakhan *et al.,* 2009).

Dry formulations of the attractant types were used inside both types of trap and they last approximately six to eight weeks (FAO, 2009).

2.2.2.3 Killing agent

DDVP (Dichlorphos strips) (FAO, 2011) were used inside each trap to kill the insects entering the trap. The DDVP strips are effective for approximately eight weeks (Manrakhan *et al.*, 2009).

2.2.2.4. Surveillance areas and trap lay-out

2.2.2.4.1 Detection survey

The area under surveillance formed part of the NPPOZA surveillance program and traps were placed in accordance with this program. Traps were placed at the Beitbridge and Pontdrift border posts which are the main ports of entry into the area, Musina town, an urban area, alongside the Musina-Weipe border fence road, the R572 and the N1 to form road transects. The area east of the N1 was not covered with surveillance traps for this study. Traps were also placed on several citrus farms in the area to cover production units (Kwasi, 2008) in addition to fruit industry production area traps as indicated in Fig. 2.2.

Higher risk areas were identified as being ports of entry as well as areas where fresh fruit and certain vegetables are confiscated and discarded from travellers who travel across borders with fruit without a permit. Areas where travellers regularly stop or overnight after entering the country such as truck stops, guest houses and hotels were also identified as high risk areas (Meats, 1998; Florec *et al.,* 2010; Vargas *et al.,* 2010). Priority was given to the Beitbridge border post as traffic can directly enter the N1 highway which is a major road connecting several cities and is continuing all the way to Cape Town. It also interchanges with other highways such as the N3 to Durban and the N4 to Nelspruit.

Musina town was chosen as a priority town as it is close to the border post and has several overnight accommodation facilities, host material growing in town as garden plants, and it is also close to fruit producing areas. The surface area of the border posts is not very large and does not contain any host trees for the placement of surveillance traps. The placement sites for traps at the border post were selected after observations such as the traffic flow through the border post to optimise trap placement (Meats, 1998).

Observations were made where trucks and passenger vehicles stop, where they discard waste and where agricultural inspections are conducted as it leads to secondary points such as dumping sites, waste collection sites (dustbins) and agricultural product confiscation bins. Trucks may often overnight close to the border and observations regarding, hotels and guest houses assisted decision making for trap placement (USDA-APHIS, 2006).

The total area under surveillance was 1100km^2. Surveillance sites were selected in parts of the area covering mostly production units where citrus is produced. The majority of the surface area was under game farming and it included the Mapungubwe nature reserve. Surveillance traps were not placed in the nature reserve and game farms except for road transect traps which were placed alongside roads passing through these areas.

2.2.2.5 Trapping density.

The trapping density required for each surveillance area type differed. The different densities for the various areas are given in Table 2.1 for the detection, delimiting and monitoring surveys. The total number of traps at the border posts and in town is dependent on the size of the areas under surveillance and feasibility to place traps in those areas. The trapping density for monitoring and verification after a pest was believed to be eradicated from an area was the same as for delimiting surveys. The ratio of Methyl Eugenol (ME) traps versus Biolure-3 component (Biolure) traps placed, were more or less five to one for all the areas except in the first km^2 during delimitation (FAO, 2009; FAO, 2011).

Table 2.1: Trapping densities for different surveillance type requirements using Methyl Eugenol (ME) and Biolure-3 component (Biolure) lures to attract *B. invadens*.

	Density at ports of entry	**Density in urban areas**	**Density along transects**	**Density in production areas**
Detection survey	3-12 ME traps/km^2	2-5 ME traps/km^2	1ME trap /10km length of transect	1 ME traps/km^2
Delimiting survey	1st km^2 block 10 Biolure +10 ME lure /km^2 2nd -4th row 2 ME lure/ km^2	1st km^2 block 10 Biolure +10 ME lure /km^2 2nd -4th row 2 ME lure/ km^2	1ME trap/2km for first 10km, 1/ 5km next 40km and 1/10km following 50km	1st km^2 block 10 Biolure +10 ME lure /km^2 2nd -4th row 2 ME lure/ km^2
Monitoring survey (eradication)	3-5 ME traps/km^2 or the same as delimiting	3-5 ME traps/km^2 or the same as for delimiting	1 ME trap 10/km^2 length of transect or the same as for delimiting	3-5 ME traps/km^2 or the same as for delimiting

2.2.2.5.1 Detection survey

At the Pontdrift border five ME traps and one Biolure trap were placed and at Beitbridge area seven ME traps and one Biolure trap. In Musina town area, four ME traps were placed not including the transect traps which cross through Musina. Selected production sites all got up to four ME traps per farm depending on the size of the area under production. Traps were placed at a density of one trap per 10 km for the extent of the road transects. A total of eighty-seven ME baited traps and twenty-one Biolure baited traps were placed in the study area (Fig. 2.2).

2.2.2.5.2 Delimiting survey.

The trap placement for the delimiting survey was done according to the detection rate and the area where exotic fruit flies were detected in the study area according to the NPPOZA action plan (Manrakhan *et al.,* 2009). After the first positive detection of *B. invadens* a 5 km zone around the trap was quarantined by the NPPOZA. The delimiting traps were set out within this area with twenty traps, ten Biolure baited and ten ME lure baited traps respectively, in the first square kilometre which formed the core area (IAEA, 2003;

Manrakhan *et al*., 2009). The first positive detections were found in an industry managed trap in a citrus orchard at the edge of the Limpopo River approximately 50km west of Musina town. Three concentric zones were set out surrounding the core area. Each zone was divided in 1km^2 blocks. Each of these blocks had two ME baited traps. Each of the zones covered an area of 8, 16 and 24km^2 as illustrated in Fig. 2.6.

2 ME	2 ME	2 ME	2 ME	2 ME	2 ME	2 ME
2 ME	2 ME	2 ME	2 ME	2 ME	2 ME	2 ME
2 ME	2 ME	2 ME	2 ME		2 ME	2 ME
2 ME	2 ME	2 ME	10 ME 10 BioL	2 ME	2 ME	2 ME
2 ME	2 ME	2 ME	2 ME	2 ME	2 ME	2 ME
2 ME	2 ME	2 ME	2 ME	2 ME	2 ME	2 ME
2 ME	2 ME	2 ME	2 ME	2 ME	2 ME	2 ME

Figure 2.6: Layout plan to place methyl eugenol (ME) and Biolure 3 component baited traps (Bio L) in a delimiting survey. Each block represents 1km2.
The red coloured centre block represents the area where *B. invadens* was first detected.

With every *B. invadens* specimen detected, the delimiting survey was extended to cover a larger area. The existing traps in the area were included when the delimiting traps were set out. The trapping was also intensified where the first *B. invadens* flies were detected for transects and a total of seventy-seven traps were placed alongside the border service road between Beitbridge and Weipe and the R572 between Musina and Pontdrift.

A total of threehundred and eighty-two ME baited traps and hundred and six Biolure traps were set out in the area (Fig. 2.2) for the delimiting survey.

2.2.2.6 Trap placement.

Trap placement is crucial to the success of any detection survey. Traps were placed as far possible in a sentinel tree, or other host tree, at least 1,5m from the ground and with as much foliage as possible covering the trap but not the entrance holes (Vayssières *et al.,* 2009a; FAO, 2011). In cases where host trees were not available, a tree as close as possible to a host tree or plant were selected. This was experienced at the port of entry as well as alongside transects (Fig. 2.5) and in some cases the urban areas (Vargas *et al.,* 2010). The objective in these areas was to detect emerging fruit fly adults from pupation after infested fruit was discarded in the vicinity of the trap. In such areas sentinel, host, or other trees were seldom available and the best alternative suitable placement area was used, taking into consideration, that the traps needed some shade and should be accessible for servicing and lure replacement (Fig. 2.7).

Figure 2.7: Chempack bucket trap placed at Beitbridge border post placed in an Acacia tree due to the absence of host trees.

2.2.2.7 Trap servicing.

The trap servicing period should not exceed the lifespan of the lures and killing strips and can therefore be within a six week period.

The objective for the detection survey was to detect fruit flies as early as possible and required that the traps were serviced at least once a month. The traps in the area were serviced every two weeks during the study period and lures and killing strips were replaced every six weeks. The content of the traps was collected at every servicing period as dead fruit fly adults deteriorate quickly (Huxham, 2002; Vargas *et al*., 2010) especially in the dry warm conditions of the Musina area. This could make identification problematic.

During the delimiting survey the servicing frequency was increased to once a week. The content of the traps for both surveys were opened and placed in bottles with 70% alcohol. Invertebrate specimens collected in sample bottles were scanned for targeted fruit flies (Manrakhan *et al.,* 2009). The content was sent to Dr. Aruna Manrakhan at Citrus Research International (CRI) for identification of all targeted fruit flies. An alert was sent out to the CRI for every suspect specimen to ensure priority identification. The identification of targeted suspect fruit flies was verified by Dr. De Meyer from the Africa Museum, Tervuren, Belgium.

The results for the detection of targeted fruit fly species were expressed as a population index in terms of the number of fruit flies per trap per day in the area (FTD). This provides a comparative measure of the population size in the area over time. It is a useful measure to compare the number of adult fruit flies captured in fixed fruit fly traps before, during and after control measures had been initiated. FTD was calculated as the total number of fruit flies captured (F) divided by the product of the total number of inspected traps (T) and the average number of days between trap inspections (D).

$$FTD = \frac{F}{T \times D}$$

An average number of days were used because the traps were serviced every two weeks during the detection survey and every week during the delimiting survey (Kwasi, 2008; Correia *et al.,* 2008; FAO, 2011). Due to the number of traps and distances apart, every trap could not be serviced at exactly the same intervals and a day or two might overlap between servicing intervals.

2.2.2.8 Trapping and servicing records.

Each trap was provided with an unique number which was recorded with the trap location (GPS coordinates in decimal degree with datum set at WGS83), trap and attractant type, servicing dates, lure replacement dates and fruit fly species captured. The records are kept on excel spread sheets but also forwarded into a central database for evaluation and data capturing purposes of the NPPOZA. Identifications received back from the CRI were later captured on the spread sheets.

2.3 Descriptive analysis

The study involved a literature study as well as a pest survey. Data collected during the qualitative pest risk assessment for risk determination was mainly of a descriptive or categorical nature that was directly tied to a more precisely quantified measure of risk. The risks that were identified during the risk assessment provided needed information to answer specific risk management questions. Several risk questions were asked and documented into subsections each with a risk rating. The risk ratings were documented in tables for each subsection. Several visits to the study area included personal interaction with producers, food processing factories and border post personnel. This provided valuable data regarding farming activities in the area, host plants produced, possible wild hosts and the movement of host products and people in and out of the area. The data collected was used to answer several of the risk questions and was formulated into risk ratings. A final conclusion was then deliberated. The literature study provided data regarding the pest biology, population dynamics, spread, host plants, invasiveness and economic damage and consequences. However, this data was collected from the pest where it occurs in other countries.

A pest survey was conducted in the Musina area over a three year period to determine the presence of the pest in the area. This supported the probability risk ratings which was determined during the risk assessment stage. Traps were set out in the area and the spatial data regarding each trap position were documented. Each trap servicing record was also documented as well as the result of the identification of each specimen. The detections were differentiated between positive *B. invadens* and non-targeted specimens. Each positively identified *B. invadens* specimen was documented in terms of the type of lure used, type of trap placement used, the vicinity of host plants and the week of the year it was detected. The number of detections was monitored and documented per week after the NPPOZA initiated an eradication program.

The results of the pest risk assessment and the pest survey were documented and used to propose new risk management options.

CHAPTER 3: RESULTS AND DISCUSSION

A. Qualitative species initiated risk analysis

3.1 Stage 1: Initiation

The methodology to determine the factors to consider for the initiation stage was described in Section 2.1.2. This constitutes the reasoning why a PRA was needed.

Pest background.

Bactrocera invadens has been detected since 2003 in Africa and has spread to over 22 countries on the continent (De Meyer *et al*., 2012). According to available literature the species was described for the first time in 2005 and caused economic damage in the countries where it was detected. It is polyphagous (Aluja & Mangan, 2008) and has adapted to attack indigenous fruit (De Meyer *et al*., 2012; Mwatawala *et al.,* 2006b; Rwomushana *et al*., 2008a).

The reasons determined for the PRA were as follows:

- This pest could be described according to the definition of a quarantine pest (FAO, 2009)
- There were indications that this pest is invasive and established quickly in several and diverse areas (EPPO, 2010)
- There was early evidence that *B. invadens* is replacing native fruit flies in their immediate range (Ekesi *et al*., 2009)
- The Musina area comprises mostly of natural vegetation and irrigated horticultural crop farming alongside the Limpopo river. Potential host plants are therefore available in the area which may serve as a first establishment point of the pest in South Africa. Once established it could spread to other production areas in South Africa
- Consultations with DAFF determined that the Beitbridge border post is a major port of entry which allows traffic from infested countries such as Zambia through to the area and the rest of South Africa (DAFF 2010, pers. comm., *May* 2010)
- Published records indicated that there is a high frequency of illegal immigrants moving from Zimbabwe to South Africa (Dumba & Chirisa, 2010)
- It was found that the pest has increased its distribution and has spread to southern Africa. The apparent southern movement of this highly polyphagous pest was the main reason to conduct a species initiated PRA for the territory and specifically for the Musina area. The Musina area indicated in this study borders Zimbabwe with the Limpopo river forming the border (Fig. 2.2)

3.2 Stage 2: Pest risk assessment

As indicated in Chapter 2.2, stage two (the pest risk assessment) was conducted using supportive information from an extensive literature review.

3.2.1 Probability of entry

3.2.1.1 Geographical distribution

Bactrocera invadens occurs in two distinct geographical areas, namely in Asia and Africa. This fruit fly has not been detected on any other continent (Khamis *et al*., 2009; EPPO, 2010).

3.2.1.1.1 Asia:

Only after specimens, which were previously collected in Sri Lanka and described as unusual variants of other species in the *B. dorsalis* complex, were re-examined, (EPPO, 2010) was *B. invadens* described as a separate species. This re-description was done after *B. invadens* was recorded in Africa. This led to the conclusion that *B. invadens* also occurred in Sri Lanka and that it could have spread from there to Africa through infested fruit. Later it was also recorded in India and Bhutan and could therefore be native to Asia (Clarke *et al.,* 2005). The occurrence of the species in India was recorded in 2005 after it was previously described as a different species collected from Tamil Nadu in mango orchards, and from Chennai (Clarke *et al.,* 2005; EPPO, 2010; De Meyer *et al.*, 2010). The recorded distribution in Asia may increase much further, as this species is similar to *B. dorsalis* and other species in the complex, which commonly occurs throughout Asia and could have been misidentified in the past during survey collections (Khamis *et al*., 2009; Tan *et al.,* 2010). The probability is also high that *B. invadens* could already have spread through trade in the Asian region to other countries (markets) due to insufficient phytosanitary measures.

3.2.1.1.2 Africa:

The distribution of *B. invadens* in Africa was mainly determined through trap and fruit surveys initiated in different countries. Many of the records were provided from unpublished sources such as CIRAD/PRPV, RID/CNEARC, IITA, ICIPE, RMCA and USDA-APHIS (EPPO, 2010). Most of these and many published records have later been referred to in other sources such as the website *Invasive fruit fly pests in Africa*. http://www.africamuseum.be/fruitfly/AfroAsia.htm (EPPO, 2010; De Meyer *et al*., 2012).

The first detection of *B. invadens* in Kenya bears no relevance to the actual time and place that this species was introduced in Africa. There could have been several introductions from Asia to different African countries from several Asian destinations (Khamis *et al*., 2009). The distribution through Africa may also not only be a result of natural spread but may be due to inter-African trade between individual countries which are not necessarily neighbouring countries and as a result of travellers transporting host material (EPPO, 2010). The worldwide distribution is illustrated in Fig. 3.1. The African countries where *B. invadens* has been detected are indicated in Table 3.1.

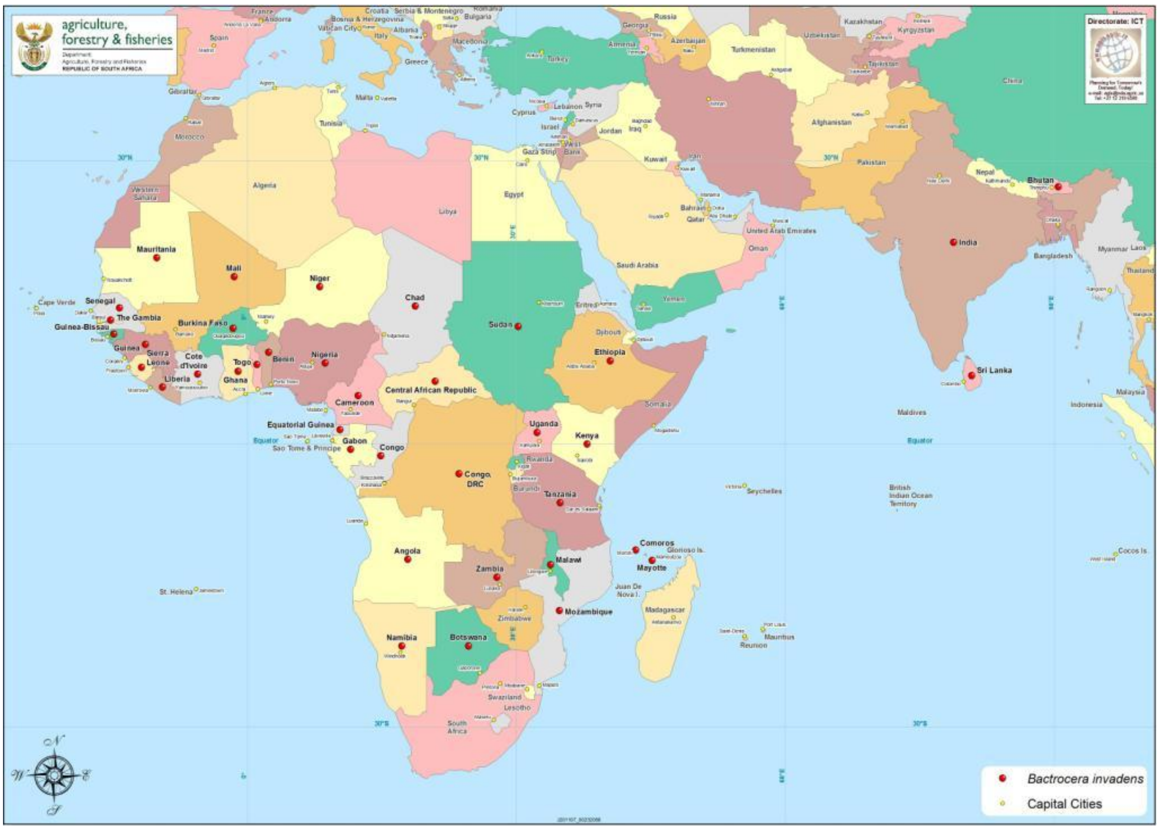

Figure 3.1: World distribution of *Bactrocera invadens.*

Table 3.1. African countries reported to be infested with *Bactrocera invadens* (EPPO, 2010; De Meyer *et al*., 2012)

Country	Date of detection
Angola	unknown/
Benin	2004-06
Botswana	2010
Burkina Faso	2005-05
Burundi	2008-11
Cameroon	2004-08
Central African Republic	2008-08
Chad	unknown
Congo	2005-11
Comoros	2005-08
Côte d'Ivoire	2005-05
Democratic Republic of Congo	unknown
Ecuatorial Guinea, Etiopia	2004-07
Gabón	unknown
Gambia	2005-06
Ghana	2004-11
Guinea	2005-05
Guinea-Bissau	2005-07
Kenia	2003-02
Liberia	2005-07
Mali	2005-06
Malawi	2010
Mauritania	2007-08
Mayotte	2007-03
Mozambique	2007-07
Namibia	2008-10
Níger	2005-08
Nigeria	2003-11
Senegal	2004-06
Sierra Leone	2005-07
South Africa*	2010-05
Sudan	2004-05
Tanzania	2003-07
Togo	2004-10
Uganda	2004-07
Zambia	2008

* Male specimens detected in Methyl Eugenol and Biolure baited traps and subsequently eradicated.

The probability of entry due to the spread and occurrence in African countries is considered to be **high**.

3.2.1.2. The availability of host plant commodities.

The possible pathways that *B. invadens* can follow to enter a new area or country where it does not occur is through hosts, commercial consignments, fruit carried by passengers and growing medium such as un-sieved compost. *B. invadens* can spread naturally from one area to another as the pest can fly and survive in wild hosts occurring in an area. In the Musina area several possible wild hosts occur such as *Sclerocarya birrea* (Marula), (Van Wyk & van Wyk, 1997) and *Harpephyllum caffrum* (wild plum), (Williams *et al.,* 2008).

This pest has been recorded on a wide range of hosts in Africa ranging from cultivated species as well as several wild hosts from several genera and plant families and is regarded as a polyphagous pest. The host range of *B. invadens* is recorded in Table 3.2. Hosts of this new invader pest in Africa were determined mainly from field records (Mwatawala *et al.,* 2006b; Kwasi, 2008; Vayssières *et al.,* 2008) after fruit had been collected in production areas in Africa (Ekesi *et al.,* 2006; Ekesi & Zillah, 2006). Fruit collection from various orchards and fields indicated that *B. invadens* similarly to the other species in the *B. dorsalis* complex has a preference for mangoes (Mwatawala *et al.,* 2006b; De Meyer *et al.,* 2010; White & Elson-Harris, 1994). Other preferred fruit are citrus, guava and bananas (Abanda *et al.,* 2008; Rwomushana *et al.,* 2008a). The status of fruit ripeness, condition or the fruit cultivars of the fruit was not always recorded for many of the hosts recorded (Mwatawala *et al.,* 2006b; De Meyer *et al.,* 2010). Not many fruit collection records were published from southern African countries. This could be important as the plant growth pattern changes from equatorial Africa towards the drier African Savannah and the southern African lowland coastal regions. Host preference studies conducted in Kenya were only done due to limited field collection and laboratory studies (Rwomushana *et al.,* 2008a). The physiology of ripening fruit differs from a fruit attached to a tree and from fruit that has been removed from a tree. It also varies between ripening phases amongst different cultivars. This may influence the susceptibility of fruit to ovipositing fruit flies (Aluja & Mangan, 2008). Choice and no-choice tests using fruit flies and various hosts have been conducted under laboratory conditions which provided some clarity regarding the preferred hosts of *B. invadens* (Rwomushana *et al.,* 2008a). However, questions remain, as several of the hosts used in this study were indicated to be conditional hosts for other fruit fly species in other studies (De Graaf, 2010) and fruit varieties and cultivars were not always documented. *B. invadens* is a polyphagous pest (Rwomushana *et al.,* 2008a) and, similar to other species from the *B. dorsalis* species complex, may have to oviposit as a result of egg pressure on any suitable surface when put under stress situations such as a laboratory. They may even oviposit on these surfaces rather than a preferred host (Aluja & Mangan, 2008).

Table 3.2. Host records of *Bactrocera invadens* recorded in Africa (EPPO, 2010; De Meyer *et al.,* 2010).

Scientific name	Family	Common name	Country recorded
Achra sapota	Sapotaceae	Sapodilla tree	Ivory Coast
Anacardium occidentalum	Anacardiaceae	Cashew	Benin,Senegal, Tanzania
Annona cherimola	Annonaceae	Cherimoya	Kenya, Tanzania
Annona diversifolia	Annonaceae	Ilama fruit	Ivory Coast
Annona montana	Annonaceae	Mountain sour sop	Ivory Coast,
Annona muricata	Annonaceae	Soursop	Benin, Kenya,Tanzania
Annona senegalensis	Annonaceae	Wild custard apple	Benin
Annona squamosa	Annonaceae	Custard apple	Kenya
Averrhoa carambola	Oxalidaceae	Carambola, starfruit	Benin,Tanzania
Blighia sp	Sapindaceae		Benin
Capsicum anuum	Solanaceae	Peppers (bell, sweet)	Tanzania,
Capsicum frutescens	Solanaceae	Chilly peppers	Benin,
Carica papaya	Caricaceae	Papaya	Benin, Tanzania
Chrysophyllum albidum	Sapotaceae	White star-apple	Benin
Chrysophyllum cainito	Sapotaceae	Star apple	Ivory Coast
Citrullus lanatus	Cucurbitaceae	Watermelon	Tanzania
Citrus aurantium	Rutaceae	Sour orange	Benin
Citrus grandis	Rutaceae	Pomelo	Tanzania
Citrus limon	Rutaceae	Lemon	Tanzania, Kenya
Citrus paradisi	Rutaceae	Grapefruit	Tanzania, Benin,
Citrus reticulata	Rutaceae	Tangerine	Benin Tanzania, Kenya
Citrus sinensis	Rutaceae	Sweet orange	Benin Kenya, Tanzania
Citrus tangelo	Rutaceae	Tangelo	Benin
Coffea arabica	Rubiaceae	Arabica coffee	Tanzania
Coffea canephora	Rubiaceae	Robusta coffee	Tanzania
Cordia sp. cf myxa	Boraginaceae	Assyrian plum	Kenya
Cordyla pinnata	Caesalpiniaceae	Cayor pear tree	Benin
Cucumis figarei	Cucurbitaceae	Hyenas watermelon	Tanzania
Cucumis pepo	Cucurbitaceae	Guard	
Cucumis sp nr metuliferus	Cucurbitaceae		Tanzania
Cucumis sativus	Cucurbitaceae	Cucumber	Tanzania, Benin
Cucurbita maxima	Cucurbitaceae	Pumpkin	Benin
Diospyros kaki	Ebenaceae	Japanese persimmon	Western Africa
Diospyros montana	Ebenaceae	Mountain persimmon	Benin
Dracaena steudneri	Dracaenaceae	Northern dragon-tree	Kenya
Eriobotrya japonica	Rosaceae	Loquat	Tanzania
Ficus sycomorus	Moraceae	Wild fig	Western Africa
Flacourtia indica	Flacortiaceae	Governor's plum	Tanzania
Fortunella japonica	Rutaceae	Kumquat	Senegal
Fortunella margarita	Rutaceae	Kumquat	Senegal, Tanzania
Garcinia mannii	Clusiaceae	Chewing stick	Western Africa
Irvingia gabonensis	Irvingiaceae	African wild mango	Benin
Landolphia sp.	Apocynaceae		Western Africa
Lycopersicon esculentum	Solanaceae	Tomato	Benin, Kenya, Tanzania
Maerua duchesnei	Cappraceae		Western Africa
Malus domestica	Rosaceae	Apple	Tanzania
Mangifera indica	Anacardiaceae	Mango	Benin, Kenya, Mozam,Senegal, Tanzania
Manilkara zapota	Sapotaceae	Bully tree	Benin
Momordica cf trifoliata	Cucurbitaceae		Tanzania

Table 3.2 continue. Host records of *Bactrocera invadens* recorded in Africa (EPPO, 2010; De Meyer et al., 2010).

Scientific name	Family	Common name	Country recorded
Musa x paradisiaca	Musaceae	Plantain	Benin
Musa sp. AAA	Musaceae	Banana	Benin, Kenya, Tanzania Uganda
Persea americana	Lauraceae	Avocado	Benin,Tanzania
Prunus persica	Rosaceae	Peach	Tanzania
Psidium guajava	Myrtaceae	Guava	Benin Cameroon Tanzania Kenya
Psidium littorale	Myrtaceae	Strawberry guava	Tanzania
Richardella campechiana	Sapotaceae	Yellow sapote	Ivory Coast
Sarcocyphalus latyfolius	Rubiaceae	African peach	Benin,
Sclerocarya birrea	Anacardiaceae	Marula	Benin,Kenya, Tanzania
Solanum anguivi	Solanaceae	Forest bitter berry	Tanzania
Solanum athiopicum	Solanaceae	Ethiopian eggplant	Tanzania
Solanum nigrum	Solanaceae	Black night shade	Tanzania
Solanum sodomeum	Solanaceae	Apple of Sodom	Tanzania
Sorindeia madagascariensis	Anacardiaceae	Sondriry	Kenya
Spondias cytheria	Anacardiaceae	Jew plum	Tanzania
Spondias mombin	Anacardiaceae	Tropical plum	Benin
Strychnos mellodora	Strychnaceae	Monkey orange	Kenya
Syzygium cumini	Myrtaceae	Jambolan	Tanzania
Syzygium jambos	Myrtaceae	Rose apple	Tanzania
Syzygium malaccense	Myrtaceae	Malay apple	Benin
Syzygium samaragense	Myrtaceae	Java apple	Benin
Terminalia catappa	Combretaceae	Tropical almond	Benin
Thevetia peruviata	Apocynaceae	Lucky nut	Tanzania
Vitellaria paradoxa	Sapotaceae	Sheanut	Benin
Ziziphus mauritiana	Rhamnaceae	Indian Jujube	Benin

Field cage studies can also be a useful tool to study oviposition in ripening fruit and cultivar preference (Aluja & Mangan, 2008; De Graaf, 2010). Field collected host data is considered valid and with data available on the time of oviposition in relation to the maturity of the fruit, less restrictive phytosanitary measures can be implemented. No such field cage studies have been conducted in Africa for *B. invadens*. Several economically important hosts of *B. invadens* are also hosts of other species within the *Bactrocera* species complex. However, in some cases there may be resistant cultivars or conditions, such as the ripeness of the fruit of those hosts which were recorded for other species in the *B. dorsalis* complex but not yet for *B. invadens*. In hosts such as avocado pear and bananas the susceptible cultivars and host condition most preferable for oviposition has been recorded for some of the other *B. dorsalis* complex species which could also be relevant for *B. invadens* (White & Elson- Harris, 1994).

A summary of some of the hosts recorded for *B. invadens, B. dorsalis* and other *Bactrocera* species in the species complex is given in Table 3.3. Hosts of several species from the *B. dorsalis* complex of species are difficult to determine, especially in areas where more than one species occurs. Several hosts have been recorded as hosts of unidentified species of the complex. Those are often unusual hosts such as coconut or a single record of some of the hosts such as *Vitis* sp. (White & Elson-Harris, 1994). Inaccurate reports can lead to faulty assumptions by importing countries regarding the actual host range of *B. dorsalis* and/or other species in the complex when a pest risk analysis is conducted. Several of the hosts which were recorded to be infested by *B. invadens* may be very good hosts or preferred hosts but the host plant is not readily available in a particular area or the fruit had been collected at a non-preferred ripening stage for the fruit fly. However, such a host may be more readily available in the importing country which may result in the establishment of the pest.

Incomplete information on host occurrence and status creates a level of uncertainty, especially in cases such as pests invading new areas. However, categorisation was done on hosts in Benin, West Africa (Vayssières *et al*., 2005; Vayssières *et al*., 2009a; EPPO, 2010; Goergen *et al.,* 2011). EPPO also categorised hosts in the pest risk assessment done for *B. invadens* according to the available data on pest and host interactions. Major and minor hosts were identified which can be used by NPPOs to determine the host status more accurately. Host preference studies conducted in Kenya determined the host status of *B. invadens* for different fruit hosts (Rwomushana *et al.*, 2008a) and could be compared with the findings in Benin. *B. invadens* has so far been frequently reared from mangoes (Rwomushana *et al.,* 2008a). It can be concluded that *Mangifera indica* cultivars occurring in Africa are a preferred host for *B. invadens* (Kwasi*, 2008;* Vayssières *et al*., 2005; Vayssières *et al*., 2009a; EPPO, 2010). This is further supported by Table 3.3 since mangoes are the only fruit crop which is a host to many of the *B. dorsalis* species in the species complex (White & Elson-Harris, 1994).

Table 3. 3. Hosts of *Bactrocera invadens* which may be found in the Musina area in comparison with other species of the *Bactrocera dorsalis* species complex.

Hosts recorded from three and more species are shaded in grey. Host records after White & Elson-Haris, (1994).

Host plant	Family	*B.carveae*	*B. dorsalis*	*B. occipitalis*	*B. carembolae*	*B. papayae*	*B. philippinensis*	*B. kandiensis*	*B. dorsalis (?)*
Capsicum anuum	Solanaceae		✓		✓	✓			
Capsicum frutescens	Solanaceae								✓
Carica papaya	Caricaceae		✓			✓			✓
Citrullus lanatus	Cucurbitaceae		✓						
Citrus aurantium	Rutaceae								✓
Citrus grandis	Rutaceae								
Citrus limon	Rutaceae								
Citrus paradisi	Rutaceae								
Citrus reticulata	Rutaceae	✓	✓		✓				
Citrus sinensis	Rutaceae		✓		✓	✓			
Citrus tangelo	Rutaceae								
Cucumis figarei	Cucurbitaceae								
Cucumis pepo	Cucurbitaceae								
Cucumis sativus	Cucurbitaceae								
Cucurbita maxima	Cucurbitaceae								
Eriobotrya japonica	Rosaceae		✓						
Ficus ? sycomorus	Moraceae								
Fortunella japonica	Rutaceae								✓
Fortunella margarita	Rutaceae								
Lycopersicon esculentum	Solanaceae		✓		✓				
*Mangifera indica**	Anacardiaceae	✓	✓	✓	✓	✓	✓	✓	✓
Musa sp.	Musaceae								
Persia americana	Lauraceae		✓						✓
Psidium guajava	Myrtaceae	✓			✓	✓			✓
Sclerocarya birrea	Anacardiaceae								
Solanum anguivi	Solanaceae								
Solanum athiopicum	Solanaceae								
Solanum nigrum	Solanaceae								
Solanum sosomeum	Solanaceae								
Strychnos mellodora	Strychnaceae								
Terminalia catappa	Combretaceae	✓			✓				
Thevetia peruviata	Apocynaceae								
Ziziphus mauritiana	Rhamnaceae					✓			

* Indicates a favourable host for various *Bactrocera spp*.

? Indicates unidentified species in the *Bactrocera dorsalis* species complex.

Due to the remaining uncertainties regarding recorded cultivars and ripening phase used in host studies (Aluja & Mangan, 2008; De Graaf, 2010) no differentiation was made in this study between the host preference for different species, varieties or cultivars. All the hosts that had been recorded from Africa were considered as a possible pathway of the fruit fly to be followed from an infested country to South Africa. The hosts comprised of fruit from indigenous plants, ornamental trees or commercial fruit trees that were naturally occurring, grown for individual use or grown commercially. This increased the risk of the pest to follow the pathway through numerous hosts and numerous pathways such as commercial fruit, passenger luggage and exotic fruit for friends and family.

The risk that this pest will spread through the availability of host plant commodities was considered to be **high**.

3.2.1.3 The probability that *B. invadens* may be introduced with commercial fruit trade.

3.2.1.3.1 Introduction from African countries.

It is unknown how *B. invadens* was introduced into Africa. Commercial fruit trade or travellers carrying infested fruit with eggs or larvae as well as soil or growing medium containing pupae were found to be likely pathways. In Africa this pest was most probably spread through informal trade and the movement of people between countries and from infested to non-infested areas.

Data on commercial consignments from African countries to Europe and the UK indicates that a high percentage of fruit intercepted was as a result of *B. invadens* infestation. French authorities' intercepted 39 consignments of *B invadens* infested fruit originating from African countries in 2009 at the Roissy airport. They identified 175 *B. invadens* specimens from infested mango fruit (EPPO, 2010). Interception data according to EPPO indicated that *B. invadens* was intercepted from various African countries, by more than one member state as illustrated in Table 3.4 (EPPO, 2010). Many of the host species associated with *B. invadens* is not commercially traded on the export market and interception data is not readily available.

Table 3.4: Interception records of *B. invadens* in fruit in the United Kingdom and Europe (EPPO, 2010).

Consignment origin	Intercepting country	Date	Number of interceptions	Commodity
Cameroon and Togo	France	2010	19	Mango
Senegal, Mali, Kenya, Burkina-Faso, Côte d'Ivoire, Togo, Cameroon	France	2009	39	Mango
Cameroon, Côte d'Ivoire, Mali, Burkina-Faso, Senegal	France	2008	18	Mango
Cameroon	Switzerland	2009	1	Mango
Sri Lanka (1) Senegal (9)	UK	2006-2010	10	Guava(1), Mango(9)
Senegal	UK	2010	5	Mango
Gambia	UK	2010	2	Mango
Ghana	UK	2010	1	Mango
Kenya	UK	2010	1	Mango

According to DAFF, Plant Health, 2009 the NPPOZA has adopted a precautionary approach and has put all commercial fruit trade on hold from countries where the pest is known to occur, until feasible mitigation options can be implemented. Trade was put on hold after the NPPOZA received official communication from an affected trading partner that *B. invadens* had established in their territory or, as a result of a scientific publication indicating the presence of the pest.

However, commercial fruit from countries where *B. invadens* has not been reported from was allowed entry into South Africa. This still poses a significant risk as surveys to detect the pest have not been conveyed in all African countries. In some countries surveys were only conducted over short periods and no permanent surveillance traps are in place. There may also be a risk that some countries do not notify the presence of the pests to their trading partners immediately. South Africa is importing fruit from African countries by air freight directly to the international airports or by road through border posts. Commercial fruit are moved into the Musina area through the Beitbridge border post which borders Zimbabwe and from other places such as the City Deep Fresh Produce market, the fresh produce marketing chain. According to DAFF, Directorate Inspections Services (DIS), commercial fruit that passed through Beitbridge might have originated from as far as Zambia (DAFF, DIS 2010, pers. comm., 13 May 2010).

The risk of introduction of infested fruit from commercial consignments from African countries to the area is considered to be high.

3.2.1.3.2 Introduction from non-African countries.

Fruit imported from Asian countries where *B. invadens* is known to occur may be infested with this fruit fly. *B. invadens* might have spread over a much wider area in Asia, which increases the risk of introduction through trade from other countries in the Asian region. Although this contributes to the overall risk of *B. invadens* to be imported into South Africa, it will not be imported directly to the Musina area as there is no port of entry which imports directly from Asian countries into the Musina area. Fruit may be distributed from fresh produce markets from the central parts of the country into the Musina area by road.

The town Musina has several supermarket retail chain stores which may import host material originating from Asian countries to their fresh produce depots from depots from the central parts of the country. *B. dorsalis* and other species within the species complex are regarded as quarantine pests. Pre- and post-harvest phytosanitary measures already apply to mitigate the risk of the other *Bactrocera* species which will lessen the probability of the pest to follow this pathway and establish in the area. However, these measures are applied for specific pests which may be less effective against *B. invadens*.

Import conditions for fresh apple and pear fruit from China exists. China is the only country bordering known infested countries from where fresh apples and pears are imported (DoA, 2007). These conditions do not include *B. invadens* as a quarantine pest as it is not known to occur in China but it does include *B. dorsalis* as a quarantine pest (DoA, 2007).

The risks of entry with fruit from Asian countries are considered to be **medium**.

3.2.1.4 Plants for planting with growing medium attached to the plants

The NPPOZA does not allow entry of commercial plants with soil attached to its roots and the risk of entry is therefore generally reduced. However, entry of private collections of pot plants from neighbouring countries is still allowed. This considerably increases the introduction risk of pupae in pot plant soil from countries such as Botswana, Namibia and Mozambique. These pot plants may also be introduced directly into the Musina area. Adult fruit flies may emerge from the pupae and start a new population in the Musina area. Plants for planting may also pose an additional risk as it may bear fruits infested with eggs or larvae, although according to DAFF, Directorate Plant Health (DPH), this is prohibited by the NPPOZA (DAFF, DPH 2010, pers. com., 26 February 2010).

The risk in terms of pot plants which are allowed entry from neighbouring countries is considered to be **high**.

3.2.1.5 Introduction through fruit carried by passengers or informal fruit trade.

Fruit carried by passengers may be the main pathway by which *B. invadens* has spread throughout Africa. Many African borders are political and therefore porous as there are no geographical or physical barriers which prevent casual movement. Informal movement of people and informal traders occurs on a daily basis through border crossings along South Africa (Peberdy, 2000).

Zimbabwe has not reported the occurrence of this fruit fly neither have they reported any official surveys. *B. invadens* has been detected in Zambia up to the southern border with Zimbabwe, in the north of Botswana, north and central parts of Mozambique and in Malawi.

Many travellers carry fruit with them when they travel internationally as a snack and it may be the cause of many fruit fly outbreaks (White & Elson-Harris, 1994). Passengers, commuters and truck drivers could potentially carry fruit with them when they enter the area from Beitbridge border post but also from other land border posts as well as through airports. Casual workers cross the border on a daily basis to work in South Africa or to do shopping. Truck drivers are crossing the Musina and Groblersbrug border posts on a daily basis from countries as far as Zambia, to deliver goods as far as the Durban port. It has been reported according to DIS, that track drivers who couriers commercial fruit that passed through Beitbridge border post, also carries fruit in the truck cabin originating from any potential source along the road(DAFF, DIS 2010, pers. comm., 13 May 2010).

The cargo may not be host material but the drivers may have food with them which was obtained from infested areas. Truck drivers need to rest frequently and truck stops occur throughout South Africa. To clear trucks with cargo is a lengthy process which can cause the trucks to stop overnight at border posts. Unwanted or rotten fruit for personal use may be discarded at overnight or resting spots. It can also be simply thrown out of a window alongside roads. Ramasodi (2008) also determined that many travellers carry fresh fruit with them when travelling to South Africa by air from countries infested with *B. invadens*. These travellers may also enter South Africa by road.

Many illegal immigrants move through the PRA area and may carry fruit with them. The fence line between Zimbabwe and South Africa was found to be cut at several places, responsible for the illegal movement of people into South Africa (Personal observation, 2009, 2010 and 2011) as can be seen in Fig. 3.2. The first detection of *B. invadens* in South Africa occurred next to the Zimbabwe border in a Methyl-Eugenol trap in a Citrus orchard about 100m from the border fence (Fig. 3.3), in close proximity from where the fence was cut.

Figure 3.2: Damaged border fence between South Africa and Zimbabwe.

Figure 3.3: Citrus orchard next to the Zimbabwe border where the first *B. invadens* specimen was detected in 2010.

Athough it is illegal to carry fresh fruit across the border without a permit (Anonymous, 1983) it is also impossible to search every vehicle or person crossing the border.

The risk that infested fruit may be carried informally to the Musina area or the rest of South Africa is considered to be **high**.

3.2.1.6 Natural spread

B. invadens has spread throughout Africa due to several possible initial introductions from Asia, informal and commercial trade, travellers and natural spread as it has established viable populations in all the countries detected so far except South Africa. The spread to the Musina area may be contributed to natural spread. If the pest is established in neighbouring countries close to the borders of South Africa the pest will spread to the Musina area, either directly from across the Limpopo River from Zimbabwe or Botswana or through the Kruger National Park from Mozambique. It may also spread from the Musina area to any other area in South Africa bordering the Musina area through natural spread provided that there is enough host material available and that the climatic conditions are favourable. The Musina area forms part of the Savannah biome (Rutherford & Westfall, 1994). The specific veld types in the area differ due to changes in topography but the majority of the area constitutes a Mopani shrub veld type (Acocks, 1988). If *B. invadens* establishes north of the Limpopo river, it would probably spread through natural vegetation to the south provided enough host material is available at a particular point in time. Many other indigenous plants may also be hosts, yet unrecorded. *Sclerocarya birrea* (Marula) and *Ficus sycomorus* (Wild fig) occurs in the area and has been recorded as natural hosts of *B. invadens* (Ekesi *et al.*, 2006). Wild figs occur on the river banks in the Savannah biome (Rutherford & Westfall, 1994), including the river banks of the Limpopo river (Fig. 3.4). Cultivated host material is also available in the form of commercial and small scale fruit produced throughout Africa.

Large citrus estates are operating next to the northern banks of the Limpopo river (CRI, CGA 2010, pers. comm. 1 June 2010) in Zimbabwe. These estates are adjacent to citrus orchards on the southern banks in South Africa and could be a point from where *B. invadens* spreads into the Musina area if regular surveillance does not occur in Zimbabwe.

Figure 3.4: Wild fig tree bearing possible host material for *B. invadens.*

The risk of *B. invadens* spreading from neighbouring countries to the Musina area and the rest of South Africa as a result of natural spread is considered to be **high**.

3.2.1.7 Import through cut branches with fruit used for ornamental purposes

This may be a new pathway which was not previously recognized and which should be followed up (EPPO, 2010). There is no information regarding the possible introduction risk of fruit on branches. A general condition of import for plant material by the NPPOZA prohibits importation of twigs and branches with fruit. The risk that *B. invadens* can follow this pathway is high if allowed or undetected, but the risk of the pathway is low. There may be a growing trend to trade with cut branches with colourful long lasting berries or fruit on it such as *Coffea arabica* and *Coffea canephora* (EPPO, 2010).

The risk of the realisation of this pathway is considered to be **low**.

3.2.1.8 Soil and growing material as a commodity.

Soil and untreated growing material from all countries are prohibited by the NPPOZA. Although it is a possible pathway for pupae to be present in the soil or growing material from infested countries, the probability of the pathway is low (EPPO, 2010).

The risk of this pathway is high if no control is implemented, but since the commodity is prohibited it is considered to be **low**.

3.2.1.9 Probability of importation

Truck drivers may move cargo and food in the cabin all the way from Lusaka in Zambia through Zimbabwe or Botswana to the Durban port. Cargo therefore moves through the Musina area. The status of the fruit carried informally by travellers or cargo conveyers is unknown as it may be purchased alongside road stalls, greengrocers or supermarkets. Fruit originating from unmanaged orchards may have a high infestation rate (Stonehouse *et al.,* 1998).

South Africa has an influx of illegal immigrants moving into the country. It is estimated that there are between 2, 5 and 4 million illegal immigrants in South Africa (Dumba & Chirisa, 2010; Solomon, 2010). Many immigrants are seeking work in the Limpopo province and are employed on farms close to the border with Zimbabwe. It is likely that the fruit fly will arrive in South Africa through host material with small informal consignments. The Musina area is especially at risk due to informal movement through the borders of Zimbabwe (Dumba & Chirisa, 2010) and the unknown status of the fruit fly in Zimbabwe. The Beitbridge border post is the only official border post between South Africa and Zimbabwe (Anonymous, 1983). This border post is also situated in the Musina area in Limpopo. There may also be facilitation by corrupt border officials to let migrants and other illegal immigrants through the border post (Dumba & Chirisa, 2010). This border post has to handle all the incoming commercial plant material. Consignments with commercial host material produced in Zimbabwe are permitted by the NPPOZA as the presence of the fruit fly in Zimbabwe is unknown and officially declared as not occurring in the country.

The risk that fruit is imported into the area is estimated to be **high**.

3.2.1.9.1 The ability of the pest to survive transport and storage.

Rwomushana *et al* (2008b) determined that the lower temperature threshold for the development of eggs, larvae and pupa for *B. invadens* is was 8.^{o}C, 9.^{o}C and 8.7^{o}C for the eggs, larvae and pupae respectively. Hallman *et al* (2011) indicated that the postharvest cold treatment schedules suitable for the treatment of *C. capitata* would also be applicable for *B. invadens*. This was confirmed by Grout *et al* (2011) during the development of a cold treatment for citrus fruit that the upper threshold of citrus pulp should not exceed 0.9 ^{o}C for 16 days.

Fruit infested by fruit fly eggs or larvae may not be detected during sorting packing and inspection (Stonehouse *et al.,* 2004) if it is done in commercial pack houses as such inspections are mainly concerned with quality standards and appearance of fruit.

Mango producers in Ghana, is comprised of small scale farmers as well as commercial farmers. The small scale farmers generally harvest mangoes at a fully ripe stage and have poor storage facilities at ambient temperature. The commercial farmers has better storage facilities but the optimum storage temperature for mangoes are at 10^{o}C (Aboagye, 2009) which is higher than the minimum larval development temperature of *B. invadens* (Rwomushana *et al*., 2008b; Grout *et al.,* 2011) It is not easy for informal buyers to detect infested fruit and fruit fly larva can survive in harvested fruit. Mangoes are also not transported at temperatures lower and 10^{o}C (Aboagye, 2009).

Long term storage for citrus in West African countries such as Nigeria is not practiced. Fruit is sold along road sides where wholesale marketers collect it and distribute it further (Fakayode *et al.,* 2010). Kenya has an increase in horticultural production which also increased on the international market in terms of exports. However, the lack of proper storage facilities may be a constrain (English, *et al.,* 2004).

Kenya has a large and lucrative avocado export industry. Avocados for the local market is harvested often at an overripe stage and packed and transported in gunny bags. Hass avocados are stored at 5 ^{o}C and ripened at 15 ^{o}C which is also above the lower development threshold of *B. invadens* (Chegeh et al., 2010).

Land-locked countries such as Zambia need to rely on road transport to move fresh produce. Zambia has an intricate network of marketers who collects fresh produce from small scale farmers, commercial farmers and neighbouring countries for the local retail shops and open markets (Hichaambwa & Tschirley, 2006). The transportation of fresh produce by road in general are seldom at a temperatures lower than 10^{o}C due to road temperatures, cooling technology applied and monitoring systems (Wilson *et al.,* 1999). Fruit may be stored and kept in retail shops at optimum temperature for storage which for subtropical fruit such as mangoes may be approximately 13-14ºC at most to avoid chilling injuries (EPPO, 2010).

The period for fruit fly development increase at lower temperatures and leads to increasing mortality at all life stages (Duyck *et al.,* 2004). Regular interceptions of *B. invadens* have occurred in mango consignments to the UK and EU of commercial consignments which suggests that the fruit fly can survive general fruit storage and transport temperatures

(EPPO, 2010). The temperature threshold of larval development of *B. invadens* further confirms that it will survive in consignments above 10 ^{o}C (Ekesi *et al.*, 2006).

The risk that the pest will survive transport and storage is estimated to be **high**.

3.2.1.9.2 The ability of the pest to survive existing pest management procedures

B. invadens is difficult and expensive to manage for the many small scale farmers across Africa. Methods such as trapping to monitor increases in population in order to implement control actions with bait sprays, or cultural methods such as orchard sanitation and burial of fallen fruit need to be implemented. Orchard pest management measures against fruit flies will help to reduce the population overall in a particular orchard. This in turn will reduce the probability of fruit fly oviposition on fruit which are to be harvested. It cannot eliminate oviposition entirely and the risk will therefore remain and increase with a lack of orchard pest management methods (Kwasi, 2008; Van Mele *et al.,* 2007; Vayssières *et al.,* 2009b).

Commercial fruit consignments introduced with strict protocols have reduced risk, but without a proper post-harvest treatment that will eliminate fruit infested with fruit fly larvae and eggs, the risk will remain high for most host commodities. Fruit flies and in particular *B. invadens* have the ability to survive general orchard management procedures, detection, transportation and storage. It has a history of interception commercial consignments. This supports the probability of the importation to be high which is increased in combination with all the other factors such as the informal movement of host material (EPPO, 2010).

The probability that live *B. invadens* individuals will arrive in South Africa with the importation of fruit hosts from infested countries is therefore estimated to be **high**.

3.2.1.10 Probability of dispersion.

After arrival in the Musina area either as adults flying into the area, or through fruit hosts or other pathways as larvae or pupae, the individual surviving fruit flies must complete their life cycles by finding new food sources and mates to reproduce.

3.2.1.10.1 Ability of *B. invadens* to disperse from the pathway to a suitable host.

The Musina area is generally a warm low altitude area with low to average rainfall. It is normally a frost free area. The probability is high that, once infested fruit is introduced into the Musina, fruit flies may develop in the area and infest other host material due to the availability of host material. Host plants of *B. invadens* grow in the Musina area as wild plants and commercially. These host plants have seasonal fruiting periods where fruit may be susceptible to ovipositing females. Some wild host plants such as *Ficus* spp. may be located closer to water sources and are not evenly scattered throughout the area. Other host plants such as Marula fruit have a short fruit bearing period from December to February. Cattle and game farms may have wild hosts but not commercial hosts such as citrus, which increases the duration of available hosts. Due to the large surface area that cattle and game farms utilise in the PRA area, there may be several months between commercial fruit production periods and wild fruit ripening periods, which could make it difficult for *B. invadens* specimens to find suitable hosts (Fig. 3.5). Fruits and vegetables produced in the area which are hosts for *B. invadens* are all irrigated and are illustrated in Fig. 3.5 as cultivated, temporary commercial irrigated land. There may be commercial and wild hosts however at farm house gardens throughout the area (personal observation, 2010, 2011) which may lead to small areas where the pest can establish. Fruit entering the area informally/illegally will be disposed in the area and fruit flies will be able to complete their life cycle if the climatic conditions are suitable (Ekesi *et al*., 2006) and if disposed close to hosts with suitable fruit. The dispersion during winter months may be less probable as wild fruit species are less available and commercial citrus, tomato and pepper production occurs in isolated pockets in the area surrounded by indigenous flora. The majority of the land is utilized for game and cattle farming. The Mapungubwe National Park and World Heritage Site is situated in the area next to the Limpopo river. From the Musina area fruit flies can disperse naturally within the area, especially alongside rivers and around fruit production areas. It can also be distributed with infested fruit within the area with fruit vendors and road side fruit stalls selling infested fruit.

The probability of dispersion is considered to be **high**.

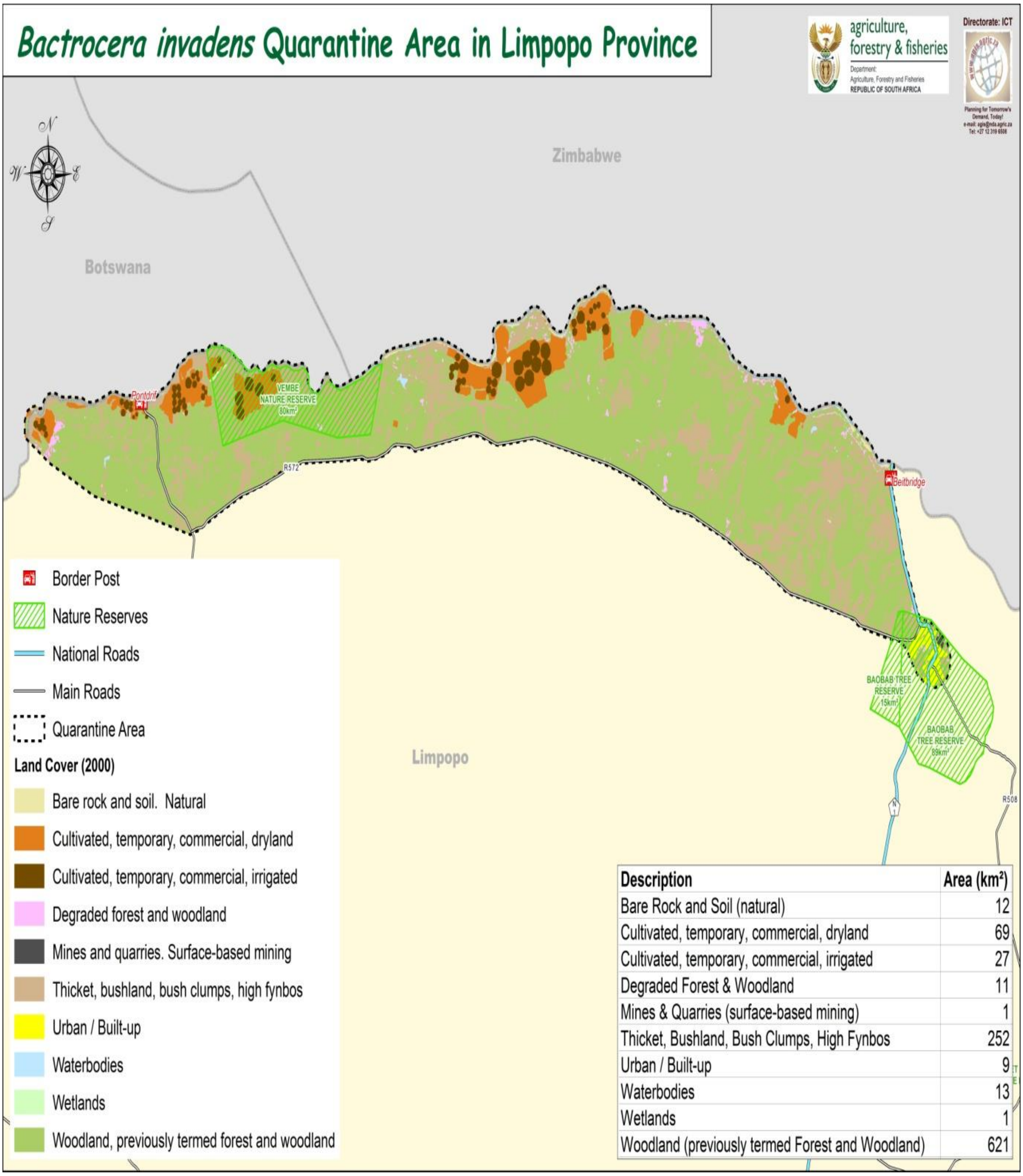

Figure 3.5: Land use data in the *Bactrocera invadens* PRA area to indicate the irrigated areas under cultivation which are used for fruit production, natural vegetation and the Musina urban area where host plants may be cultivated or grown.

3.2.1.10.1 Distribution of the imported commodity in the PRA area.

Mangoes, citrus, tomatoes peppers and cucurbits are the majority of commercial host plants grown in the area. Vendors crossing the border may be selling these and other possible hosts locally on an informal basis, especially in the more populated areas in Musina. The town of Musina up to Beitbridge was scouted by driving on the N1 and walking every street of the town. Several street vendors were selling host material in Musina town next to the main road (N1) which carries most of the traffic. There were also several informal vendors at the border post and at informal settlements in the area. Host material such as citrus, papaya, guava, mango and tomatoes are also cultivated in home gardens and the produce could be distributed amongst friends and family in the area and outside the area. Ornamental plants which are host plants for *B. invadens* such as *Eriobotrya japonica* or Loquat were also found at house gardens. Murula trees are commonly grown in house gardens, on sidewalks at schools and other open areas in town. These hosts may also be carried outside the area to be sold in other South African towns or along roadsides. Imported commodities may be sold either by street vendors or supermarkets in the area. The distribution in the area is limited as Musina is the only town in the area.

The risk of distribution of imported material in the area is considered to be **medium**.

3.2.1.10.2 Risks from by-products and waste from infested hosts in the area

Eggs may develop into larvae within fruit throughout the distribution chain. Fruit infested with eggs and possibly first instar larvae may not be initially detected. Consumption may lead to detection of infestation and the discarding of the infested fruit in waste.

Infested fruit with second and third instar larvae may be detected by consumers before and during consumption and would be discarded. Unwanted fruit or waste products may be discarded at municipal garbage dumps, alongside roads, in backyard compost heaps or on farm dumpsites.

A vegetable packing house and a tomato processing plant operate in the PRA area. The vegetable packing house packs locally grown salad tomatoes, peppers and small cucurbits which can be infested host material. The freshly packed produce is distributed to major fresh product markets in South Africa. The packing facility has a retail outlet which sells packed vegetables that may contain host material directly to the public. The packing is preceded by quality checks during sorting. The tomato processing plant has a sorting process. Produce with blemishes, marks or signs of decay will not be packed but either be discarded or sold to pig or cattle farmers or sent back for composting. Produce showing signs of decay will not be processed into tomato sauce and will be discarded by selling it to livestock farmers. Most of the tomatoes for processing are produced in the area and are not transported outside the area (ZZ2 tomato farms; Tiger Brand/ All Gold tomato processing plant in Musina 2010, pers.com. 1 July 2010).

The risk of fruit fly to be introduced into the PRA area through waste and by-products as a result of processing is considered to be **low.**

The risk of fruit fly able to spread within the PRA area as a result of the movement of infested host material from an infested production unit to a packing or processing facility is **high** if waste material such as peels and pulp is discarded in the area.

Risks from by-products and waste from infested hosts in the area is considered to be **medium.**

3.2.1.12 Risk summary: Probability of entry.

The probability of entry as determined by investigating different factors is indicated in Section 2.1.2.1. The risk rating for each of the factors is indicated in Table 3.5.

Table 3.5 Risk rating for all the factors considered to determine probability of entry

Risk factors considered	**Risk Rating**
Geographical distribution	High
The availability of host plant commodities.	High
The probability that *B. invadens* may be introduced with commercial fruit	High
Plants for planting with growing medium attached to the plants	High
Fruit carried by passengers or informal fruit trade	High
Natural spread	High
Cut branches with fruit used for ornamental purposes	Low
Soil and Growing material as a commodity	Low
Probability of importation	High
Probability of dispersion	High
Risks from by-products and waste from infested hosts in the area	Medium
The probability of entry	**High**

The probability of entry of this polyphagous fruit fly pest through the pathways identified from various areas and distribution within the area after introduction is regarded as **high.**

3.2.2 Probability of establishment.

3.2.2.1 Availability of suitable hosts in the PRA area.

Citrus and mangoes are cultivated commercially in the area as possible major hosts together with tomatoes, peppers and cucurbits such as pumpkins, melon and watermelons. However, the host crops mostly cultivated in the area are citrus and tomatoes (Producers meeting Weipe area 2010, pers. comm., 1June 2010; DAFF, 2010a). Guavas, papaya and bananas species are grown commonly as garden plants throughout the area including urban areas (Vegetable producers meeting Weipe 2010, pers. comm., 10 June 2010).

After personal communication with individual producers in the area, during a farmers meeting held at one of the farms, the total estimated commercial production area in hectares for fruit and vegetable hosts of *B. invadens* in the Musina area (by calculating individual farmer host production) was approximately 2500ha in 2010. This was further supported by a land use data map as illustrated in Fig. 3.5 as irrigated land. The total hectares will differ between seasons due to crop rotation and other cultural practices. Citrus production is the most intense all along the Limpopo river together with tomato production. Mangoes are produced to a lesser extent and mainly for dried fruit and to preserve. Host material is produced close to water sources to enable irrigation. Intercropping systems and crop rotation occurs generally in the area. Citrus may be produced together with mangoes, tomatoes and peppers. Farmers that prefer cash crops in the area may allocate land for several cash crops depending on market value, time of the year and their management of crop rotation systems. Several non-host fields are also found in between host fields, such as maize, cotton and potatoes which are usually irrigated. Some irrigation fields may be laid barren in between host orchards.

South Africa is known as a highly diverse country with several biomes which may consist of fruit bearing trees and shrubs suitable to a polyphagous fruit fly (Rutherford & Westfall, 1994; Acocks, 1988; Van Wyk & van Wyk, 1997). Crop production in the PRA area is mostly directly adjacent to the Limpopo River as the river is needed for irrigation. The PRA area also consists of natural vegetation on the river banks, in between irrigated areas and in areas with game and cattle farming activity. This includes the areas south of the R572 as irrigation for crops is not possible due to the distance from the river (Fig.3.5).

The commercially produced host crops, closer to the river, consisted of orchards and circles with host material produced at high density. Pest control of several pests, including naturally occurring fruit flies is also conducted in the area for various crops within the normal crop integrated management systems (IPM). Natural hosts in the area together with high density crop production alongside the river may contribute to the establishment of *B. invadens* should it be accidentally introduced in the area. The probability of a variety of other host plants being grown in private gardens for personal fruit use is also high. This includes the urban areas of Musina town, around Beitbridge border post and on farm house gardens which includes those of the game and cattle farms. The Musina town was scouted and fruit grown in private gardens included citrus, mangoes, avocado and papaya. Vegetables grown in the town included cucurbits and tomatoes. A complete host plant survey should include all the private gardens and has not been conducted. Host plants grown as ornamental plants may be less managed in terms of fruit fly control than those grown for fruit production.

Bactrocera invadens has not necessarily been exposed to all possible hosts in Africa and is enlarging its host range through the region. South Africa is a major producer of stone, pome fruit and table grapes. According to SA Fruit farms (http://www.safruitfarms.com/table-grapes.aspx) stone and pome fruit are not produced on a commercial scale in the Limpopo province, but there is commercial production of table grapes in the province. Several citrus cultivars are produced in the area, but mainly *Citrus paradisi* and *Citrus reticulata*. Citrus fruit is harvested from May to September. Mangoes are harvested from November to January. Tomatoes are grown throughout the year and can serve as alternative hosts. Host plants are therefore available throughout the year in the Musina area.

The probability of establishment in terms of the availability of suitable host plants in the area is considered to be **high**.

3.2.2.2 Pest control measures applied in the area.

Pest control programs of existing local fruit fly populations and other insect pests may influence the establishment probability of a new pest entering the area. As fruit fly eggs are oviposited underneath the exocarp of the fruit hosts they are considered to be internal pests of fruit. The development occurs inside fruit and the larvae are not affected by normal contact sprays. Existing insecticidal spray regimes may not have any impact on the establishment of *B. invadens* on crops such as tomato and peppers as no control against local fruit flies is applied in the area. Integrated pest management programs are generally applied against fruit flies on citrus and mango production as well as cucurbits (including melons and watermelons). Commonly occurring fruit flies in the area are species such as *Ceratitis capitata* (Weidemann) (Mediterranean fruit fly), *C. rosa* Karsh (Natal fruit fly), *C. cosyra* (Walker) (Marula fruit fly) (Annecke & Moran, 1982), *Dacus bivittatus* (Bigot), and *D. ciliates* Loew (White& Elson-Harris, 1994). The *Ceratitis* species may be oligo- or polyphagous but *Dacus* species are strictly oligophagous specialising on cucurbits (White & Elson-Harris, 1994).

Citrus, mango and cucurbit producers in the area have fruit fly management programs as part of their production control systems. This includes the use of the monitoring of local fruit flies and protein bait spray or male annihilation programs (Citrus research International (CRI) 2010, pers. comm., 31 May 2010). Tomatoes and peppers are recorded hosts for *C. capitata* (White & Elson-Harris, 1994) that does occur in the area, but has not been considered to be a pest of tomatoes in South Africa (ZZ2 farms 2010, pers. com., 30 June 2010). Insecticide spray programs in tomato and pepper fields for other pests may contribute to unintentional fruit fly control such as *C. capitata*. This is a known pest on tomatoes (White & Elson-Harris,

1994) in other countries but has not been detected in the fruit cut surveys conducted in the area, where 1138 tomato fruit were sampled in 2010. Currently, trapping measures are used throughout South Africa for the detection of *Bactrocera* species (Barnes & Venter, 2008).

There are no official area wide pest management programs implemented against *Ceratitis* or *Dacus* fruit fly species in the PRA area by the NPPOZA and the introduction of specimens of *B. invadens* in areas with wild host plants would therefore not be controlled by control programs such as area wide bait sprays, if not detected.

Fruit fly control programs through sanitation and bait-sprays alone do not aim to eradicate fruit flies from an area but aim to reduce the population levels to acceptable production levels or in terms of technical compliance with export programs.

The current pest control programs in the area can therefore not prevent establishment but can reduce the risk of establishment in citrus and mango production units. The risk of establishment in natural areas where wild host plants occur apart from areas where pest control programs are applied remains to be **high.**

3.2.2.3 The reproductive strategy and survival of the pest.

The lifespan of adult fruit flies differs between species but they can live for several months. This would differ within different environmental conditions. When adults of *B. dorsalis* are exposed to cool climatic conditions and enough food they can live up to 12 months (EPPO, 2010). However, *B. dorsalis* prefers warm humid weather for optimum reproduction when primary hosts such as mangoes are available. Most *Bactrocera* species have a nominal torpor temperature threshold of between 2 to 7 ºC (EPPO, 2010). *Bactrocera invadens* have mean development time ratios for immature stages of between 75.74 and 17.76 days, between 15 and 30 ºC, respectively (Rwomushana *et al*., 2008b). The lower development threshold was 8.8°C for eggs, 9.4°C larvae and 8.7°C for pupae. The short development period at average temperatures of above 20 ºC as the mean generation time for *B. invadens* was determined to be 30.7 days at 28 ± 1º C (Rwomushana *et al*., 2008b).

The establishment rating in terms of reproductive strategy under average temperature of above 20 ºC is therefore **high**.

3.2.2.4 Suitability of the environment.

Cold temperatures and low relative humidity are considered to be the most important abiotic parameters that would affect *B. invadens* establishment (EPPO, 2010; Rwomushana *et al*., 2008b; Vayssières *et al*., 2009a). Because the species continues to spread in Africa, the limits of its climatic tolerance are not yet known. It is also not known how well this fruit fly is adapted to micro climatic conditions such as within a citrus orchard or at a river bank environment. Musina is a warm to very hot area and forms part of the Savanna biome (Rutherford & Westfall, 1994). The area has an annual rainfall of above 235 mm with a large range of temperature minima. Severe frost can occur for short periods in the winter months within a period of 120 days. Long summers of six to seven and a half months occur. It has a Summer Aridity Index (SAI) of less than 4.0 (Rutherford & Westfall, 1994). The 30 year climatic average of the Musina area indicates that it is an area which experiences maximum temperatures of 44 ºC in summer and can reach a minimum of -4 ºC in winter. The average maximum rainfall is 339mm per year (SA Weather Service, 2011) as indicated in Table3.6.

Table 3.6: Weather data of the Musina area.

Climatic information of the normal values, according to World Meteorological Organization (WMO) prescripts, based on monthly averages for the 30-year period 1961 – 1990 (SA Weather Service, 2011).

Month	**Temperature (° C)**				**Precipitation**		
	Highest Recorded	Average Daily Maximum	Average Daily Minimum	Lowest Recorded	Average Monthly (mm)	Average Number of days with >= 1mm	Highest 24 Hour Rainfall (mm)
January	44	34	21	13	58	7	102
February	41	32	21	13	57	7	79
March	41	32	20	10	39	5	112
April	40	30	16	6	27	3	92
May	38	27	11	1	10	2	42
June	32	25	7	-4	4	1	44
July	33	25	7	-3	1	0	6
August	38	27	10	-4	1	0	19
September	42	30	14	4	12	1	44
October	42	31	17	8	24	4	43
November	43	32	19	11	49	6	74
December	43	33	20	11	57	6	66
Year	**44**	**30**	**15**	**-4**	**339**	**42**	**112**

A climatic prediction analysis performed with CliMEX software by the European Plant Protection Organisation (EPPO) indicated that the northern Limpopo area may be suitable for establishment (EPPO, 2010) in irrigated areas and falls at the end of the potential establishment range (Fig. 3.6). The EPPO pest risk analysis indicated that Africa as a whole is suitable for establishment.

According to De Meyer *et al.,* 2010, South Africa may especially be at risk for establishment due to suitable climatic conditions in the low lying coastal areas. In their study they used two corresponding models to determine the potential global distribution for *B. invadens*. Instead of using the CliMEX model they used ecological niche models (ENM) for their study. This is partly due to the irregularity of data from the known ranges in which this pest occurs, as it is a new pest with limited available data from research work. Occurrence data from parapheromone traps are used rather than from fruit hosts (De Meyer *et al.,* 2010). Two correlative techniques at default settings were used, namely, GARP a genetic algorithm technique and Maxent a maximum entropy method (Fig 3.7). Both models predicted the Musina area as a suitable area for establishment, although it falls at the end of the predicted range (De Meyer *et al.,* 2010). The most suitable areas for establishment would be the equatorial high rainfall areas but the fruit fly is also well established in savannah areas with low or no winter rainfall.

It is postulated that the available data used in these studies is incomplete and that the fruit fly may be able to establish further as predicted due to monoculture production of fruit and the availability of host material closer to water sources. The general population build up is considered to be lower in Savannah areas than in high continuous rainfall areas but establishment is possible especially in areas with monoculture production and irrigation (De Meyer *et al.,* 2010; EPPO, 2010).

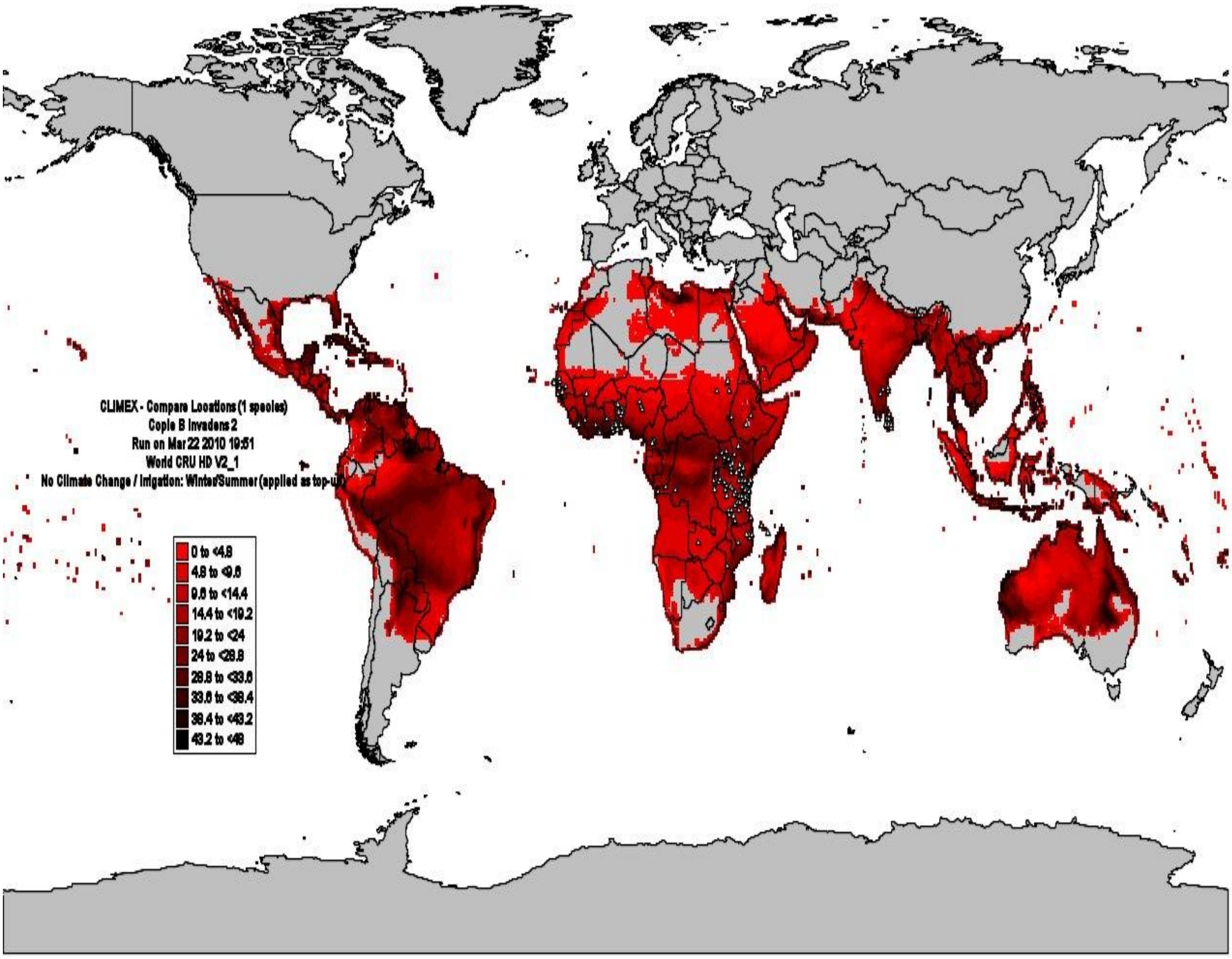

Figure: 3.6 The CliMEX model conducted by EPPO indicating potential distribution of *B. invadens* in the world including the possibility of irrigation as that would increase host plant prevalence (Ecoclimatic index)(EPPO, 2010). Darker colours indicates higher possibility.

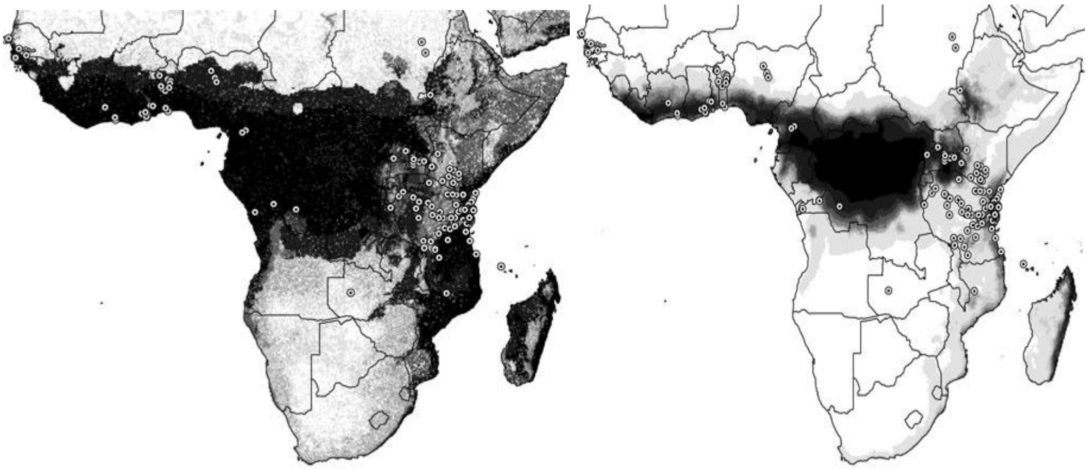

Figure 3.7: The two models Genetic Algorithm for Rule-set Prediction (GARP) on the left and the maximum entropy method (Maxent) on the right indicating predicted distribution of *Bactrocera invadens* in Africa and Madagascar on a grey scale. White, predicted absence, as indicated by the LTPT thresholding; shades of grey indicate higher levels of prediction (chosen arbitrarily), with black the highest strength for predicted presence (De Meyer *et al.,* 2010).

Establishment in South Africa as a whole is predicted to be the highest in eastern coastal areas and subtropical areas especially when host material is produced at high intensities. Establishment potential alongside the Kwazulu Natal coast to Eastern Cape is predicted to be high according to all three models (De Meyer *et al.,* 2010; EPPO, 2010).

The risk to establish in areas within South Africa outside the study area such as the Levubu, Thohoyandou and Letsitele areas is predicted to be **high**.

It is therefore predicted that *B. invadens* would be able to establish in the Musina area but in very low population numbers. The risk rating for the Musina area taking into consideration the suitability of the environment but to include the irrigated areas which increases host fruit production in the area is therefore considered to be **medium**.

3.2.2.5 Risk summary for the probability of *B. invadens* to establish.

The risk factors and ratings are summarised in Table 3.6.

Table 3.6: Risk rating for all the factors considered to determine a risk rating for probability of establishment

Risk factors considered	**Risk Rating**
Availability of suitable hosts, in the PRA area	High
Pest control measures applied in the area	High
The reproductive strategy and survival of the pest	High
Suitability of the environment	Medium
Overall probability of Establishment	**High**

The probability that fruit flies will establish within the Musina area and in South Africa is **high**.

3.2.3 Probability of spread.

3.2.3.1 The suitability of the natural or managed environment for natural spread.

Bactrocera invadens has established in humid lowland regions of Africa (Hanna, 2005), as well as dryer savanna regions of Zambia (EPPO, 2010). It also occurs from east to west Africa on the equatorial belt as far south as northern Mozambique (Correia *et al.,* 2008; Goergen *et al.,* 2011), northern Namibia (EPPO, 2010), Malawi (SADC 2010, pers. comm., 25 October 2010) and northern Botswana (IPPC, 2010). Fruit crops are commercially grown, and natural hosts occur in the Musina area especially in close proximity of river banks. It is therefore likely that *B. invadens* would spread from one host plant to another. The fruit flies in this genus are good flyers and can disperse from one production unit (orchard or field) to another. As these fruit flies are internal borers they can easily be spread inside host material aided by human transport throughout and outside the area to other fruit producing areas in South Africa with suitable climatic conditions for *B. invadens* to establish. Hosts are therefore readily available.

3.2.3.2 Presence of natural barriers

The presence of natural barriers such as semi-deserts or mountain ranges may inhibit long range natural spread of these fruit flies. However the Musina area is not bordered by any visible natural barrier. The long distances between commercial host crops in South Africa may reduce the potential for long-range natural spread of fruit flies.

Bactrocera adults engage in extensive dispersive movements during the early adult phase prior to host-seeking and mating, and mature flies leave locations where hosts are dwindling in search of new hosts. During these periods, some individuals may move long distances in a few weeks. A dispersal distance of 25 km has been recorded for *B. zonata* (IAEA, 2000). Should these fruit flies be introduced to major commercial production areas of South Africa they may be capable of unaided short distance spread.

Such studies have not yet been completed for *B. invadens* but it might be possible for a polyphagous species to spread unaided from the Musina area to the rest of Limpopo to Mpumalanga through to Kwazulu Natal to the Eastern Cape Province and eventually to the Western Cape.

The risk of spread due to the absence of natural barriers is considered to be **high**.

3.2.3.3 Potential for movement with commodities or conveyances

The most probable and quickest way for fruit flies to spread is through infested fruit from one area to another. Although the NPPOZA has published regulations regarding the spread of fruit from an infested area with *B. invadens* to a non-infested area, it would be almost impossible to regulate this on a continuous basis. The informal trade (hawkers, fruit stalls etc.) of fruit in South Africa is well developed. Informal traders purchase fruit directly from farmers and sell it elsewhere in the area and the rest of South Africa. This is a lucrative form of trade simply known in the Musina area as the “bakkie trade” which requires further investigation (Producers meeting Weipe area 2010, pers. comm., 1July 2010). It makes it very difficult to obtain any real data on how much produce is actually traded in this way. The citrus fruit produced in the Musina area is packed inside or in some cases outside of the area and sent to Durban for export to other countries. Citrus fruit not destined for export is sent either to Polokwane to a juice factory or sold on farm. Other crops such as tomatoes, peppers and cucurbits produced in the area are mostly moved to fresh produce markets and retail outlets outside the area to the centre of the country if sold fresh. The City Deep fresh produce market takes most of the produce from this area. From there it is sold to retail outlets all over the central parts of the country. Many farmers sell only to small scale vendors on their farm and get market related prices without bearing the transport costs themselves (Producers meeting Weipe area 2010, pers. comm., 1 June 2010). Farmers loose fruit through the illegal harvesting of fruit for personal usage. The town Musina and surrounding area use fruit trees as garden plants partly as a supplementary food source or as ornamental plants and these fruit are often sent to friends and family elsewhere in South Africa.

The NPPOZA has started a national exotic fruit fly surveillance project in 2006 and all the major cities, ports of entry (or exit); major roads and production areas have traps suitable for the early detection of *B. invadens.* The early detection, available action and response plans will help to prevent the further establishment and aid in the possible eradication of the fruit fly if detected in the country.

The probability that infested fruit may be moved out of the PRA area after establishment is considered to be **high.**

3.2.3.4 Overall probability of spread.

The risk factors and rating to determine the overall probability of spread is summarised in Table 3.7.

Table 3.7. Summary of the overall probability of spread.

Risk factors considered	**Risk Rating**
The suitability of the natural or managed environment for natural spread	High
Presence of natural barriers	High
Potential for movement with commodities or conveyances	High
Overall probability of spread	**High**

The probability that fruit flies will spread within South Africa, based on a comparison of factors such as availability of hosts, host movement and natural dispersal is considered to be **high**.

3.2.4 Overall probability of entry, establishment and spread.

The polyphagous nature, good natural dispersal abilities of *B. invadens* and the formal as well as informal marketing of fruit within South Africa together with suitable climate plus the availability of hosts will support a high rating for this fruit fly's ability to spread from one area to another as indicated in Table 3.8.

Table 3. 8: Risk ratings for the probability of entry establishment and spread

Risk factors considered	**Risk Rating**
Probability of entry	High
Probability of Establishment	High
Probability of Spread	High
Overall probability of entry, establishment and spread	**High**

The overall probability that fruit flies will enter, establish and spread into and within Musina and the rest of South Africa is considered to be **high.**

3.2.5 Potential economic consequences.

South Africa is a major producer of fruit for export. Most of the country's fruit production is destined for the export market. Between 2008 and 2009 South Africa had a gross farm income from horticulture of about 3.5 billion USD (DAFF, 2010 (a)). Horticultural production increased by 2.6 % from 2009 to 2010. Prices of fruit products increased by 11 % while prices of vegetables decreased by 10% (DAFF, 2010 (a)). Producers of horticultural products showed a gross income increase of 4, 4 % in 2010, from R33 945 million in 2009 to R35 449 million in 2010. The citrus producers showed an increase of 40 % amounting to R6 455 million. This was a result of improved sales and better prices realised on export markets.

Vegetable producers suffered a decrease in income of 1,1 %, of R12 577 million. South Africa experienced a drop of 15,4 % in net farming income on a year to year basis for all products after the deduction of all production expenditure over the same period. However, the consumer price index of vegetables increased by 2,0 %, from 154,7 to 157,8 and that of fruit increased by 4,0 %, from 137,4 to 142,9 (DAFF, 2010a).

Export data from Trade Map for 2010 valued code 08 products (edible fruit, nuts, peel of citrus fruit, melons) at R638 billion (Trademap, 2011) citrus fruit (fresh and dried) was valued at R95 billion. Citrus fruit is therefore a major contributor to the export market in South Africa.

3.2.5.1 Production of hosts in the Musina area in South Africa.

The production of the major fruit crops in the Limpopo province is illustrated in Table 3.9. This was retrieved from Statistics South Africa for the 2002 year (SA Statistics, 2002). The Musina municipal area of 75 7700ha is in the Vhembe district. The Musina municipal area which extends beyond the study area produced host material of *B. invadens* over an area of 5000ha in 2002. Approximately 2500ha of host material was produced in the study area (Fig. 3,5) in 2010 (Producers meeting Weipe area 2010, pers. comm., 1July 2010).

Table 3.9: Crop production data in the Musina area and Limpopo.
The highest as well as the total production volumes are highlighted in grey.

	Musina		**Limpopo**	
Crop	**Ha**	**Metric tons**	**Ha**	**Metric tons**
Tomatoes	859	45874	4970	224661
Cucurbits	162	1931	3137	46498
Oranges	3950	76437	18634	439556
Grapefruit	5	72	1017	34107
Mangoes	72	889	5361	33020
Total	**5048**	**125203**	**33119**	**777842**

SA Statistics, 2002

.

Citrus is a major crop produced in the Musina area destined mainly for export. During the 2008/09 production season the citrus industry contributed R5.8 billion to the total gross value of South African agricultural production. Lesser quality fruit which did not qualify for the export market was sent to the fresh produce markets or for juicing. Approximately 7.53 % of all the citrus produced in South Africa were sent to a national fresh produce market (DAFF, 2010(b)).

Citrus production in Limpopo province is divided into three areas, namely, the Limpopo area, Letsitele area and Hoedspruit area. The Limpopo area stretches along the Limpopo River in the west up to Musina including the Tshipise area about 30 km south east of Musina (CGA 2011, pers. comm., 7 July 2011). The total area under production in the province was reported to be 18 146 ha in the Citrus Growers Association's (CGA) annual report of 2010 and represented 32 % of the country's citrus production. This data is mainly retrieved from CGA members and does not reflect the area under production from all the citrus growers. The Limpopo province is a major contributor to the total of citrus planted in South Africa with 11 677ha of valencias, 623ha of soft citrus and 3 312ha of grapefruit planted in 2009 (DAFF, 2010(b)).

The citrus industry is labour intensive and employs more than 100 000 people. The majority of the workers are employed in picking and packing houses and approximately a million households depend on the South African citrus industry in one form or another (DAFF, 2010b).

Limpopo province represents 66% of national mango production. Most of the mango plantings in Limpopo are found in the Soutpansberg, northern, central and southern Letaba as well as Hoedspruit regions. Although mangoes are cultivated to a lesser extent in the study area, the possibility is high that it will increase when more lucrative export markets for South African mangoes open. Mangoes are mainly produced for processing either dried fruit or atchar from this area (DAFF, 2010c).

Tomatoes are the second most consumed vegetable crop in South Africa besides potatoes and it contributed 19% to the gross value of vegetable production in 2009 (DAFF, 2010d). The Limpopo province is a major production area for tomatoes with 50 % of the total area planted to tomato production in South Africa. During 2009, approximately 3590ha was produced in the province with the northern Lowveld at 2700ha and the far northern areas of Limpopo at 890 ha (DAFF, 2010d). Tomatoes were mainly produced for processing and for the fresh produce market. Tomatoes were mainly processed for canning, freezing, dehydration and juice production. A small quantity was exported to the SADC region. The Limpopo province exported tomatoes to the value of R244 373. Tomatoes produced for the fresh produce market originated from an area close to Pontdrift and an area close to Musina town. The tomatoes produced for processing were also sold on farm as fresh produce depending on existing market prices or, otherwise sold for canning or tomato puree or sauce (Farmers meeting 2010, pers.comm. 1 June 2010) in the area. Most of the tomatoes for processing were sent to a processing factory in Musina. The rest was sent mainly for canning or juice outside the area. The total percentage of volume allocated for processing in 2009, was 25% which was valued at R151 million (DAFF, 2010d).

3.2.5.2 Economic consequences as a result of the loss of export markets.

The introduction of *B. invadens* can disrupt foreign market access severely. The Limpopo province produces fresh citrus and avocado fruit for the export market. The Musina area produces citrus fruit destined for the export market. As *B. invadens* has only been described in 2005, the full distribution of the pest in the eastern hemisphere is not yet known. It was only reported from Bhutan, India and Sri Lanka in Asia. It has not been reported from any country in the pacific, North or South America, Middle East or Europe. South Africa exports fruit to China, Taiwan, Russia, Iran, Israel, Japan, South Korea, Europe, United Kingdom and the United States of America and several African countries. South Africa already comply to strict phytosanitary measures imposed on the trade of deciduous, citrus and fresh table grapes with regard to *Ceratitis capitata* and *C. rosa*. Citrus from the Limpopo valley area which includes the Musina area is exported to the EU, South Korea, China, Japan and Russia.

The presence and the uncontrolled spread of *B. invadens* in Africa caused the United States of America to issue a Federal Order regarding this pest which entails that fruit exports from an infested country will be prohibited unless the country can prove that the areas of export are free from this pest or an acceptable treatment is mitigated (USDA-APHIS, 2008). If *B. invadens* should establish in the Musina area, the risk is high that a temporary ban of citrus from South Africa will be imposed until the NPPOZA can prove that the areas designated to export to the USA are free of *B. invadens*.

The total citrus volumes produced for export in 2009 was 1 928 718 tons (CGA, 2010). The value of citrus exported from the Vhembe district was R13 2070 000 and the whole of the Limpopo province of R98 0359 000 (CGA, 2010).

The existing citrus export program to the USA includes a strict post-harvest inspection pre-clearance program conducted by the NPPOZA as well as USDA-APHIS inspectors and a compulsory post-harvest cold sterilisation program for quarantine fruit fly pests such as *C. capitata* (DoA, 2008).

No post-harvest treatments for *B. invadens* existed until quite recently when cold sterilisation tests were successfully conducted on *B. invadens* in Kenya and Austria (Grout *et al*., 2011; Hallman *et al.,* 2011; Ware *et al.,* 2012). The tests were conducted *in vitro* and *in vivo* and it was found that the cold treatment schedules commonly used in trade for various commodities were effective against larvae of *B. invadens.* The studies were conducted on *B. invadens* larvae in infested citrus fruits (oranges), whereby a post-harvest cold treatment of 0.9°C or lower for a consistent period of 16 days was recommended (Grout *et al*., 2011) and a treatment of 1.5°C for not less than 18 days(Ware *et al.,* 2012). However, no trials for post harvest treatments for *B. invadens* control was done on other commodities and warm-water and vapour heat tests were inconclusive (Hallman *et al*., 2011).

Japan as well as South Korea requires a pre-clearance inspection and cold treatment of citrus export fruit against fruit flies of quarantine importance such as *C. capitata* and *C. rosa*. The cold treatment imposed by South Korea is -0,6±0,6°C or lower through pre-cooling, and maintained (-0,6±0,6°C or below) for 24 days which is effective against *Thaumatotibia leucotreta* (Meyrick) (False codling moth), Lepidoptera, Tortricidae. The USDA cold sterilisation period also covers false codling moth sterilisation. The USDA has recently published a cold treatment schedule for citrus fruit against *C. rosa, B. invadens* and *T. leucotreta*, treatment no T107-k (USDA, 2011). The schedule provides a strict guideline that the fruit must be treated for 24 days at -0.5º C or below. If the temperature reaches a high spike of -0.27ºC it will be increased to 8 hours and be nullified if the temperature exceeds 1.11 ºC for more than 48h (USDA, 2011). This treatment has not been negotiated with South Africa and can thus not be implemented in the case of a *B. invadens* introduction (DAFF PH 2011, pers.comm. 6 July 2011). It is not suggested on any other fruit type nor is there any differentiation regarding the citrus species or cultivars. This temperature schedule may result in an increase in freezing of fruit and high losses (Citrus Research International (CRI) 2011, pers.comm., 8 April 2011). Japan specifies the treatment period for fruit flies as 12 consecutive days maintained constantly at -0,6±0,6°C or below. Japan also imposed a post harvest inspection by NPPOZA inspectors in Japan for the duration of the export season. Pre-harvest fruit fly monitoring for *C. capitata* is part of the work program with South Korea and an IPM program for *C. capitata* with China (DoA, 2006).

The European Union (EU) has a zero tolerance for non-European fruit flies and would by detection of a single *B. invadens* larvae in fruit reject the consignment. Senegal, Benin and Ghana have suffered economic losses due to *B. invadens* interceptions (EPPO, 2010) with regards to consignments that were rejected by the EU on arrival after interception of *B. invadens*.

The risk that South Africa will lose export markets for produce such as citrus after the introduction or establishment of *B. invadens* is considered to be **high**.

3.2.5.3 Economic consequences as a result of the loss of production and the increase of production costs.

Throughout Africa *B. invadens* has caused huge losses to countries, producers and individuals. Mango is the major crop affected and is produced on subsistence, small and commercial level in infested countries (Mwatawala *et al.*, 2009). Crop loss has been estimated on a few occasions and quantitative data is only available on mangoes and citrus in a few occasions (EPPO, 2010). The small and subsistence farmers do not necessarily use pesticides or cultural sanitation to control local fruit flies and suffer as a result of large losses. The cost of controlling *B. invadens* can escalate as this pest constitutes an additional environmental risk (Ekesi *et al.,* 2006). The estimated losses of *C. cosyra* and *B. invadens* on mangoes can be from 10 % in the beginning of the season up to 80 % towards the end of the season, with late cultivars being infested the most (EPPO, 2010). This will differ between cultivars, production methods and duration of the season. In West Africa (Benin) losses were calculated at 15% in early April and 69% in mid June. This can be as much as 6.5 tons per hectare (Vayssières *et al.*, 2008).

It has been documented that several citrus species and cultivars have been heavily infested by *B. invadens* in East and West Africa such as *Citrus limon, C. reticulata* and *C. sinensis* (Rwamushana *et al*., 2008; Vayssières *et al.,* 2008). The level of infestation of *B. invadens* differs depending on the host species cultivar, existing fruit fly population levels, production methods and agro- ecological zone (EPPO, 2010). High levels of fruit fly damage were recorded on citrus fruit in South Benin. Up to 98,3% of all fruit flies that emerged from citrus fruit between 2008 and 2009 was *B. invadens.* It is the most destructive fruit fly pest of citrus in South Benin with infestation rates calculated in terms of pupae developed per kilogram of fruit. It ranged from 3 pupae per kg on sweet orange to 25 pupae per kg on mandarins. The recorded losses for *Citrus reticulata* Blanco (Mandarins) was up to 22%, *Citrus sinensis* (Sweet orange) to 25 per kg in sweet orange and *Citrus tangelo* (Tangelo), 34 % (EPPO, 2010).

Citrus, mangoes and cucurbits are grown in the Musina area and are also hosts for specific *Ceratitis* and *Dacus* spp. Fruit fly control programs, such as the regular application of protein bait sprays are already in place during fruit production. This consists of an organophosphate and a protein lure or Spinosad (Spinosyn A, D) and protein hydrolysate lure such as GF120 Naturalite (GF1111). In commercial citrus orchards a weekly sanitation program is followed by removing all fruit that has dropped to the ground to shred it or to feed it to game. Shredded fruit pulp is spread in the sun between the rows to dry (Producers meeting Weipe area 2010, pers. comm., 1June 2010).

Tomato production in the Musina area occurs throughout the year for the fresh produce market, for a tomato sauce factory in the area and for canned tomatoes (Tiger Bran/All Gold 2010, pers. comm., 1 July 2010; Producers meeting Weipe area 2010, pers.comm., 1July 2010). Currently no fruit fly control actions are used for tomato production in South Africa as fruit flies are generally not considered as a production problem. It may also cause production problems on tomatoes in the area if left uncontrolled. This will lead to an additional production cost to the producer. Tomatoes are harvested every day and once in fruiting stage it is almost impossible to apply pesticides. Most often depending on market prices, tomatoes which were produced for processing are sold as fresh tomatoes from local farms directly to informal traders. These producers may suffer additional losses if tomatoes are not treated against *B. invadens*.

The risk that the establishment of *B. invadens* will lead to additional economic losses for the producers in terms of production losses if left uncontrolled and in terms of additional control cost is considered to be **high**.

3.2.5.4 Economic consequences as a result of environmental impact.

Although it is not possible to calculate environmental impact at this stage it needs to be noted that the specific relationship of fruit flies such as *C. cosyra* (Marula fruit fly) with its host *Sclerocarya birrea* (Marula) may not be fully understood. It was shown that *C. cosyra* an indigenous fruit fly species was rapidly displaced by *B. invadens* at Nguruman Kenya only four years after the first detections of *B. invadens* (Duyck *et al*., 2004; Ekesi *et al.,* 2009). *Ceratitis cosyra* occurs commonly in Africa as well as in the study area. *B. invadens* is therefore not only of commercial value but it can be considered to be an invasive species to the environment (De Meyer *et al.,* 2010).

There is evidence of a mutually beneficial relationship between certain *Bactrocera.* species with orchid species from the genus *Bulbophyllum* as the orchid flowers excrete a synomone which attracts males for pollination (Tan & Nishida, 2000; Tan *et al*., 2006). The synomone methyl eugenol is a strong attractant to many *Bactrocera* species and the metabolites act as strong sex pheromones (Hee & Tan, 2004). Area wide control of fruit flies in Malaysia, where both the orchids as well as some of the economically important fruit flies occur, such as *B. papaya* is a concern due to the important ecological role these fruit flies play in their natural environment (Tan & Nishida, 2000). However, such symbiotic relationships are not documented for *B. invadens.*

As determined in Kenya, *B. invadens* is an invasive insect that can displace indigenous fruit fly species. The consequences on natural ecosystems are considered to be **high**.

3.2.6. Overall consequences

The risk factors considered to determine the economic consequences are summarised in Table 3.10. The consequences of a new pest into the agricultural system may lead to an increase in production costs. The risk that increased production costs will have a negative economic effect and consequences on the agricultural sector in South Africa is high.
The risk that the potential suspension or loss of export markets for host material such as citrus fruit may have a negative economic effect on such an industry is therefore high.

Table 3.10 Risk consequences and ratings

Risk factors considered	Risk Rating
Economic consequences as a result of the loss of export markets	High
Economic consequences as a result of the loss of production and the increase of production costs	High
Economic consequences as a result of environmental impact.	High
Overall consequences.	**High**

The economic and environmental consequences that the establishment of *B. invadens* in South Africa will have are considered to be **high.**

3.2.6 Overall assessment

The probability of *B. invadens* being introduced into the Musina area is high. Several possible pathways for introduction were identified which increased that risk. The Musina area produces many of the known host plants for *B. invadens* and succession of fruits from suitable hosts are available throughout the year but, alternative hosts such as wild fruit is sparsely dispersed. Multiple hosts increase the probability that *B. invadens* can disperse within the area. According to the results of three climatic modelling techniques, the Musina area has a suitable climate for the establishment of *B. invadens* but may be restricted next to rivers. However, if small populations establish in the Musina area it can easily spread to the other areas in South Africa which have a more suitable climate for establishment. This increases the risk of establishment since the areas identified in the climatic models as suitable for establishment are also large fruit production areas. These areas include areas such as the Letaba and Levubu, areas in the Limpopo province as well as the Mpumalanga fruit production areas (Fig. 3.7). Although general fruit fly control methods are applied in the area against *Ceratitis* and *Dacus* fruit flies, the current management methods would not prevent the establishment of *B. invadens*. The short reproductive cycle and high fecundity increases the risk of this fruit fly pest to establish. Once established in the Musina area it can easily spread with host commodities to the rest of South Africa.

The Musina area produces fruit and vegetable host crops for the export market, processing and for the fresh produce market. Although the majority of the land use surface is not utilised for horticulture or seasonal vegetable crops, significant production alongside the Limpopo river occurs. The consequences of establishment in South Africa may result in the loss in market access and in additional crop losses.

Although recommendations were made on available information, there are still uncertainties which must be considered. Uncertainties regarding the host status of many of the identified hosts remain, especially as resistant cultivars and ripening times can have an influence on pest management practices. The status regarding the presence of the pest in several countries in Africa as well as Asia remains uncertain as no permanent surveillance practices or survey results are available. Post-harvest treatment studies are still ongoing and the pulp temperature tolerances are not clear. These uncertainties should be addressed with further research. However, the uncertainties also increase the risk with regard to this pest, especially within an international trade environment.

The probability of introduction, spread and establishment is considered to be high for *B. invadens* with regard to the Musina area and the rest of South Africa. The consequences that this pest may have on the economy of the area and the rest of South Africa are considered to be **high**.

The overall risk is the probability (high) x consequences (high) = **High.**
The overall risk of *B. invadens* is considered to be **high**.

3.3 Specific survey for the detection and delimiting of *B. invadens*.

3.3.1 Results.

The purpose of the detection survey was to determine the status in terms of the ISPM No. 8 *Determination of pest status in an area*, of the IPPC in the study area of *B invadens* (FAO, 2009*)*. As the survey addressed the objectives of the NPPOZA, only exotic fruit flies were regarded as targeted species. The results were therefore recorded in terms of targeted species and non targeted species over a two year period. Methyl-Eugenol baited traps were considered to be a strong attractant to a specific range of *Bactrocera* species. None of the quarantine pest species attracted to Methyl Eugenol is known to occur in South Africa.

Only non-targeted species were detected in 2009. However, on 5 May 2010 the first *B. invadens* specimen was detected in a fruit industry Methyl- Eugenol baited trap. This immediately triggered an emergency response and a delimiting survey was initiated.

The delimiting survey resulted in the detection of 19 *B. invadens* male specimens from May 2010 to July 2010, in the study area. Eighteen of these were caught in methyl eugenol baited traps and one in a biolure-3 component trap as indicated in Table 3.11.

Table 3.11: Trap catches of *Bactrocera invadens* males in different targeted areas over a 13 week period between May and September 2010.
Methyl Eugenol (ME) and Biolure three component (Biolure) baited traps were used.

B. invadens males detected in 2010	Week	Lure type	Trap area type
1-3	19	ME	Production
4-9	20	ME	Production, Transect, Delimiting
10-15	21	ME	Production, Delimiting
16	23	ME	Production
17	24	ME	Production
18	28	ME	Production(home garden)
19	31	Biolure	Transect

The calculated FTD value for *B. invadens* for week 19 to 20 after the first detection was 0.00595. This FTD value was calculated during a period before any control measures were used against *B. invadens* in the PRA area.

The calculated FTD value for *B. invadens* for week 21–22 after delimitation traps were placed, but before control measures were implemented was 0.000878.

The calculated FTD value for *B. invadens* for week 23-31 after delimitation and after control measures were implemented was 0.000130.

During 2011 another five fruit fly specimens were detected in five methyl eugenol traps between May and August 2011. On 6 June delimiting traps were again placed in the area after the first detection on 25 May 2011. The delimiting traps revealed two more specimens on 15 June 2011. Two other fruit fly specimens were detected on 25 June and on 29 June 2011. The NPPOZA initiated an eradication program in the area on 7 July 2011 which included aerial bait sprays and the placement of MAT blocks. No more detections occurred in the area for the remainder of 2011. The calculated FTD value for *B. invadens* from week 24 -26 in 2011 for the area was 0.000744.

3.3.2 Discussion.

The invasive fruit fly *B. invadens* has rapidly expanded its range in Africa from 2003 to 2008. From 2008 onwards it was first detected in the more southern regions in Africa, namely Namibia, Mozambique and Zambia. This fruit fly quickly utilised the available hosts in Africa and adapted to feed on a number of wild hosts. The NPPOZA increased the existing exotic fruit fly surveillance program activities to detect the fruit fly as part of an early warning system. This was made possible by increasing the surveillance efforts with the interaction of the major fruit industries and to increase the total number of traps by adding to transects laid out in areas such as Musina and in more production places. The Musina area borders Zimbabwe and to a lesser extent Botswana. During 2009 no exotic fruit fly specimens were detected but after the first fruit fly was detected in May 2010 a rapid delimiting trapping response and quarantine resulted in the delimiting area to be identified which lead to the implementation of control measures (Manrakhan *et al.,* 2011). It is possible that the 19 specimens detected over the winter months in the Musina area represented a small localized population. The origin could have been from fruit which was moved into the area across the border with passengers or with illegal immigrants which crossed the border fence. The first detection was in a ME baited trap about 100m from the border fence. The fence in that vicinity was cut at several places, big enough for people to cross. Several detections later were close to the Limpopo River but also close to the border fence road and the R572. The R572 is used by long distance cargo trucks and passenger vehicles travelling through neighbouring countries to South Africa from as far afield as Zambia and Malawi. The road forms a link between the Pontdrift and Beitbridge border posts and is also the most southern part of the delimited area. It is possible that people travelling over large distance may have carried infested fruit with them which might have been discarded in the Musina area. Another possibility was that the fruit fly spread through natural dispersal across the Limpopo into South Africa. Botswana reported the detection of *B. invadens* in 2010 after the winter months in the northern Chobe districts. However, no reports on the occurrence of *B. invadens* were received from Zimbabwe. Natural movement alongside the river beds of the Shashe and Limpopo rivers may be another explanation as there may be enough fruit bearing trees alongside the river beds to sustain small populations.

The emergency actions of the NPPOZA initiated an eradication program through the implementation of the male annihilation technique (MAT) and the bait application technique (BAT) together with strict field and orchard sanitation. The male annihilation programme consisted of ME baited fibre board insecticidal blocks which were placed at high frequencies across the area. The bait application was implemented by ground and cover protein bait sprays (Manrakhan *et al.,* 2011). Only 19 specimens were detected over 13 weeks but there could have been more as the control measure might have had a major effect on the existing population. Additionally, the population was also subjected to biotic forces such as the Allee effect and abiotic forces such as the local weather over the period of detection. The 19 specimens detected might have been too low to sustain a population. No female fruit flies were detected in any of the Biolure baited traps. As males might have dispersed in different directions, the chances to find females out of a low local population became low. Thus the abundance of adult fruit flies became low. According to Liebhold and Tobin (2008) the Allee effect refers to a decline in population growth rate with a decline in abundance.

The detection of *B. invadens* in the area in 2010 after zero catches in 2009 and the sharp decrease after control measures were implemented is supportive of the notion of a small initial founder population. The very low FTD values since the detection of the first fruit fly support this. However, the fruit flies were detected over an area stretching 100km from west to east alongside or close to the Limpopo River but also close to and alongside the R572 and the border fence road.

The detections of five more male specimens of *B. invadens* in 2011 in the study area indicated that there was a reintroduction. No *B. invadens* detections occurred in the study area between week 31 in 2010 and week 22 in 2011 which means there were no detections for 42 weeks in the area.

This indicated that several possible pathways might have been the origin of the infestation and there might have been several introductions at the same time. The probability is high that there may be repeated introductions in the Musina area of *B. invadens* in future.

CHAPTER 4: MANAGEMENT MEASURES

4.1 Introduction

Several pathways were identified with different risk ratings in Chapter 3. The risk assessment determined that the overall probability is high for *B. invadens* to enter the Musina area and the rest of South Africa with a high probability that it would have serious economic consequences.

The territory of South Africa is regarded as a *B. invadens* free area by the NPPOZA and incursions of *B. invadens* would lead to the initiation of eradication measures (Manrakhan *et al.,* 2009).

During the detection survey which continued from 2009 to 2011 for this study, a small founder population of *B. invadens* was detected in the study area. Specimens were detected in surveillance traps over a period from May 2010 to July 2010, which was successfully controlled by the NPPOZA. The source of the founder population, or the pathway the first specimens followed to enter the area, could not be determined. However during 2011, *B. invadens* was again detected in the study area. Exotic fruit fly incursions cannot be regarded as isolated, non repeatable incidences.

Three of the pathways with a high risk rating as determined in Section 3.2.1.5 and 3.2.1.6, namely, fruit carried by passengers, illegal immigrants or informal traders (hawkers) and the natural dispersal into the country across the border from an established population from Zimbabwe or Botswana could have been the source of the incursion.

Although the number of specimens of *B. invadens* detected during 2010 and 2011 indicated very small populations, the probability that more specimens would enter via the same pathway is high. Undetected founder populations could establish and increase their abundance which would make control more difficult with serious economic consequences.

This chapter suggests additional measures for the control of *B. invadens* as a result of the outcomes of the pest risk assessment. These include both preventative and management measures.

Deployment of detection traps forming part of a continuous surveillance program ensured early detection of *B. invadens* in 2010. The affected land user was informed and delimiting traps were set out within one week after the first detection. A quarantine area was set with a

5km radius from the detection point. The quarantined area also affected several other land users. Cooperation from land users was achieved through a series of meetings held with the local farming community, technical experts and members with executive decision making powers from the fruit industry bodies namely, Citrus Growers Association (CGA), Citrus Research International (CRI), Hortgro, Fruitgro, Subtropical Fruit Association (Subtrops), Alternafruit and the South African Table Grape Industry (SATGI). Eradication started as soon as stockpiled agricultural chemicals were sent to the area. They were stored on site for implementation according to the action plan developed previously (Manrakhan *et al.,* 2009). Awareness materials were developed and distributed to inform travellers at ports of entry.

The successful control of *B. invadens* could also be a result of the inability of the organism to establish successfully due to unfavourable climatic conditions. A very low number of specimens were detected in 2010 as well as 2011, even after the delimiting traps were placed. This may be indicative of a population struggling to establish because it has reached the end of its establishment range as indicated in section 3.2.2.4.

The results of the risk assessment and the detection of *B. invadens* in the study area as reported in Chapter 3 indicate that amendments should be made to the existing import regulations for host commodities. Border control actions should also be optimised for *B. invadens* to prevent further introductions. National control actions and measures were developed from existing measures for other fruit fly species and with the information available for this pest (Manrakhan *et al.,* 2011).

4.2 Existing regulations and role player forums.

Importation of host material of *B. invadens* into the country is subject to a permit with import conditions in terms of the Agricultural Pests Act (Anonymous, 1983). Movement of host material out of an area in which *B. invadens* was detected is subject to a permit in terms of R110 (Anonymous, 2009). Once a single *B. invadens* specimen is detected in an area, a 5km radius surrounding that infestation point is placed under quarantine according to the action plan (Manrakhan *et al.*, 2009).

The quarantine area is established and demarcated by the NPPOZA. Instead of publishing a demarcated area the quarantine area is sustained by notifying each land user which produces host material of *B. invadens* within the quarantine area with an official order in terms of section seven of the Agricultural Pests Act. Such an order is a legal document which prohibits the movement of host material from the quarantine area except via a permit.

The order also describes measures to control the pest in the affected area and to prevent the further spread of the pest.

The NPPOZA has a zero tolerance for *B. invadens* and the phytosanitary measures applied to allow trade would therefore have to reach the appropriate level of protection (FAO, 2009; FAO/IAEA, 2011). The current import regulations of NPPOZA prohibit the import of host material from countries with known infestations of *B. invadens* or from areas where the pest occurs. The current interactions between the role players are illustrated in Fig. 4.1.

The NPPOZA has developed good and strong relations with the major fruit industry organisations such as Citrus Growers Association (CGA), Citrus Research International (CRI), (Hortgro, Fruitgro), Suptropical Fruits Association (Subtrops), South African Table grape Industry (SATI) and Alternafruit. This provided good links with commercial farmers which strengthened the cooperation between commercial farmers and the NPPOZA within the Department of Agriculture Forestry and Fisheries (DAFF).

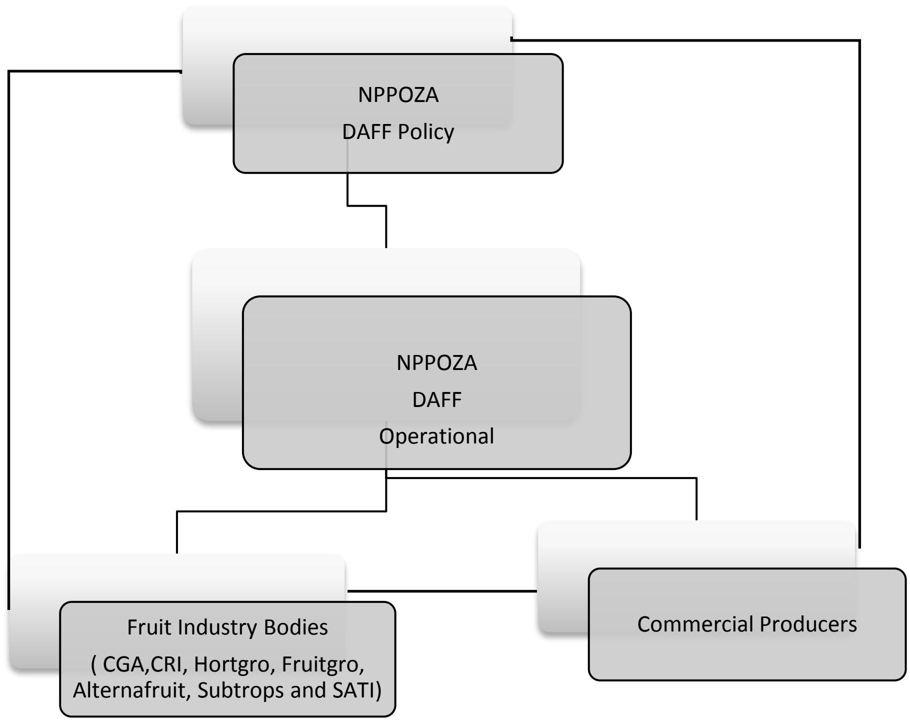

Figure 4.1: A flow chart illustrating major role players when control measures are initiated after *Bactrocera invadens* fruit fly detection.

The South African *B. invadens* action plan (Manrakhan *et al.,* 2009) is a good example of where role players and the NPPOZA developed specified actions to jointly control a specific pest.

This plan may be an oversimplification especially with regards to trade with African countries where the exact status of the pest is not known. Not all countries conduct pest surveillance and not all countries have continuous pest surveillance programs in place. The targeted pest survey conducted in the Musina area indicated that the area which was presumed to be free from *B. invadens* could be infested at any time.

The existing measures do not provide for additional options which will provide an equivalent level of protection (FAO, 2009). The existing action plan focuses strongly on commercial farming practices and does not make provision for actions to be considered when a detection of *B. invadens* occurs in residential areas and in natural vegetation and or conservation areas.

Although the principles of area wide control were implemented through the action plan very little engagement took place with local subsistence and small scale farmers. Fruit fly control on an area wide basis needs additional support and control from all government sectors up to municipal district level.

The relations with industry role players were developed historically to develop additional market access to increase export volumes of fresh fruit. The Market Access Working Group (MAWG) addresses phytosanitary and quality issues regarding market access to new markets and to maintain existing markets and involves high level representatives from all the major fruit industries, marketing agents, Perishable Products Export Control Board (PPECB) and the NPPOZA. The Phytosanitary Risk Forum (PRF) addresses phytosanitary risks of specific pests regarding existing and planned import programs and new pest introductions. The forum consists of expert representatives from the major fruit industries, importers and the NPPOZA.

A specialist group, the *Bactrocera invadens* Steering Committee (BiSC) was created in 2008, consisting of executive members of all the major fruit industries, NPPOZA and fruit fly experts. The objective of this committee is to address specific pest management problems of *B. invadens*. The committee focussed strongly on the commercial sector and national governance. Very little engagement took place on provincial and local governance levels (DAFF, DPH 2011, pers. comm., 18 May 2011). Currently industry members are involved with the NPPOZA through structured meetings as illustrated in Fig. 4.2.

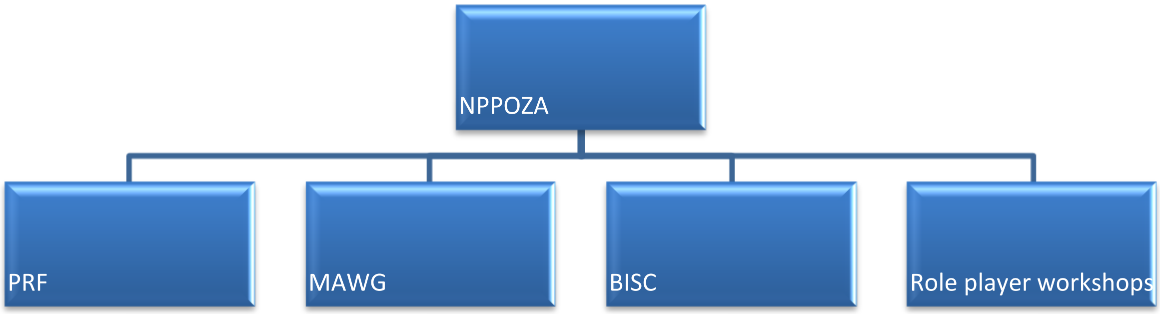

Figure: 4.2 The National Plant Protection Organisation of South Africa (NPPOZA) established working groups and forums to ensure that market access is attained and maintained in terms of existing and emerging pests. These are the, Phytosanitary Risk Forum (PRF), Market Access Working Group (MAWG) and *Bactrocera invadens* Steering Committee (BiSC).

The current action plan determines that after the detection of one specimen of *B. invadens* a delimiting survey should be conducted according to the trapping densities as indicated in Annex 1 of ISPM26 (Manrakhan *et al.,* 2009; FAO, 2009). The delimiting survey will determine the boundaries of the affected area and contributes to the area which should be quarantined.

After the detection of a second specimen of *B. invadens* in the delimited area, the area should be delimited according to the location where the second or subsequent specimens were detected. The delimited area will form the quarantine area and is regarded as the affected area (FAO, 2009). The status of the country regarding the targeted fruit fly species has changed and should be reported by the NPPO according to ISPM No. 8 (1998), *Determination of pest status in an area* and ISPM No. 17 (2002), *Pest reporting* (FAO,2009). The status of the pest reported to trading partners in 2010 from May to August 2010 was transient actionable and under eradication in accordance to ISPM No.8. Control measures were implemented after the second fruit fly was detected in a surveillance trap. This occurred according to ISPM No. 26 (FAO, 2009).

4.3 Recommended management measures: Phytosanitary import regulations.

The pest risk analysis indicated that the pest can spread through informal, formal and through natural vegetation from one country to another. The exact distribution of *B. invadens* in countries such as Botswana, Zambia and Zimbabwe is not known. This required additional measures to compensate for the risk. The IPPC provides standards which can assist with the development of import requirements to provide least restrictive measure but would still be able to ensure an acquired level of protection for the NPPO (Quinlan, 2002).

4.3.1 Pathway 1: Commercial importation of host commodities from countries where *B. invadens* is known to occur.

The differentiation and acceptance of the areas with different population levels of the fruit fly is subject to surveillance, regulatory control and management measures which should be applied by the exporting country. Additional data should be acquired by the NPPOZA from the exporting countries to enable the NPPO to develop different but equivalent options for the importation of different host commodities from different countries.

Surveillance data from permanent surveillance traps to detect *B. invadens* would provide reliable information regarding the presence of this pest in specific areas as well as an indication of the population levels when the number of fruit flies per trap per day is calculated. This will provide data for the establishment and recognition of the different types of areas recognised by the IPPC, namely, pest free areas, areas of low pest prevalence as well as pest free places of production and production sites free of pests (FAO, 2009). These areas would be developed specifically for *B. invadens*. Depending on the population level of *B. invadens* in a particular production area of host material a systems approach can be followed to mitigate the risk to an appropriate level of protection for importation into South Africa (FAO, 2009).

4.3.1.1 Pest free areas

The IPPC defines a pest free area as *"An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained"* and an area as : "*An officially defined country, part of a country or all or parts of several countries*" (FAO, 2009).

The development of a **pest free area** in a country where *B. invadens* occurs, can be achieved through intensive and permanent surveillance programs, the implementation of strict phytosanitary conditions to eradicate the pest from a dedicated area or to develop buffer zones surrounding the pest free area where the population levels are kept low through the application of phytosanitary measures. A pest free area could also be declared as a result of an area in an infested area which is geographically isolated from the infested areas (FAO/IAEA, 2011).

ISPM No. 26 (2006), *Establishment of pest free areas for fruit flies* (Tephritidae), provides such guidelines to exporting countries. Based on the information presented by the exporting country the NPPOZA can accept the declaration of a pest free area based on no detections in the area after 12 months of continuous surveillance (FAO, 2009). This cannot be accepted if the exporting country is not implementing control measures to prevent the movement of infested fruit into the pest free area.

The definition of a **pest free place of production** is a *"Place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period"* and a **pest free production site** as *"A defined portion of a place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period and that is managed as a separate unit in the same way as a pest free place of production"* (FAO, 2009). International standards exist for all the types of free areas but the management differs to a certain extent as demonstrated in the different standards. Pest free places may be an isolated farm or orchard within a generally infested area, and a production site may be a smaller unit such as a production tunnel for tomatoes which can be established within an infested area but with a buffer zone surrounding it and physical control due to the structure of such a production tunnel (FAO, 2009).

The importation of host material from countries where *B. invadens* is established into South Africa can be permitted if the management measures applied by the exporting country comply with the standards set by the IPPC of an accepted pest free area, place of production and production site.

It is therefore recommended that these are considered as additional options to be used by the NPPOZA for the importation of *B. invadens* host material from an infested country. It can also be utilised as part of a systems approach (FAO/IAEA, 2011).

The maintenance of any of the types of pest free areas would require the absence of the detection of the targeted pest in the defined area over a defined period. This will entail that the status of the pest free area is lost when *B. invadens* is detected and that trade could be suspended. The loss of status would require corrective measures which need to be implemented by the exporting country to ensure the reinstatement of the pest free area.

A pest free area for fruit flies will lose its status when two or more fertile adults were detected in surveillance traps, or a single inseminated female, or an immature specimen of the target fruit fly was detected from a fruit cutting survey or during export or import inspections (FAO, 2009). This could also be implemented for *B. invadens*.

The specific measures suggested must therefore include a detection survey protocol, delimiting survey protocol with corrective actions and control measures which are implemented by the exporting country. Surveillance records must be submitted to the NPPOZA on a regular basis by the exporting country.

These measures should be supplemented with a production unit traceability system, preclearance inspection in the exporting country by the NPPOZA, inspection before or during packing and an inspection on arrival.

4.3.1.2 Areas of low pest prevalence

An area of low pest prevalence can be defined as *"An area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures"* (FAO, 2009). Such areas can be established for *B. invadens* to form a buffer zone surrounding a pest free area or as part of a systems approach to reach the required level of protection for South Africa.

Areas of low pest prevalence are established and recognised based on surveillance records and the implementation of phytosanitary requirements for the targeted fruit fly species. This will require appropriate usage of detection traps as indicated in appendix one of ISPM No. 26 (FAO, 2009). The prevalence level is determined based on the population indicator generally used over a specific time namely the number of fruit flies per trap per day (FTD).

The results of the pest risk assessment showed that the risk that *B. invadens* will follow the pathway which could lead to establishment in South Africa if host material is imported, is high.

No differentiation is made according to the fruit fly's prevalence towards specific hosts. Once a required fruit fly per trap per day (FTD) level is determined for a specific host from a specific area, an area of low pest prevalence can be used as a mitigation measure for the importation of host material. This would not be a standalone measure and should be used together with another independent measure in a systems approach (FAO/IAEA, 2011). Imports from primary hosts from an area of low pest prevalence can be utilised in combination with a post-harvest treatment (FAO/IAEA, 2011).

The area of low pest prevalence will be suspended after the required predetermined level of low pest prevalence is exceeded for the target fruit fly species.

The exporting country may be required to temporarily suspend trade from such an area and should instate corrective actions to retain the required level of fruit flies per trap per day for an area of low pest prevalence (FAO, 2009).

The acknowledgement of an area of low pest prevalence is recommended when it is managed by the exporting country as a buffer zone surrounding a pest free area, pest free place of production or site of production. It can also be acknowledged when more information on specific host conditions required for oviposition is obtained and host categorisation has been efficiently established.

4.3.1.3 Systems approach

A systems approach can be defined as *"The integration of different risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of protection against regulated pests"* (FAO, 2009).

The development and general requirement of a systems approach is described in ISPM No. 14 (2002), *The use of integrated measures in a systems approach for pest risk management* (FAO, 2009). This can be used very effectively to manage the risk of specific hosts against the introduction of fruit fly species (Quinlan & Ikin, 2009). The use of a systems approach can provide additional assurance when used in combination with a post-harvest treatment, especially when population levels in a specific area are high (Jang *et al.,* 2006).

Each measure used in a systems approach aims to contribute to the reduction in risk that female fruit flies would oviposit on fruit selected for an export program (Mangan *et al.,* 1997).

These measures can be applied in a pre-harvest period, during harvesting and during a postharvest period including transport (Quinlan & Ikin, 2009). Pre- as well as post-harvest measures are essential to develop systems approaches for fruit flies and can be developed by utilising existing integrated pest management (IPM) measures and area-wide pest control measures as phytosanitary measures. This contributes to reduce the risk of the fruit fly pest being present during the post-harvest phase in conjunction with other mitigation options to reduce pest population levels in the area (Jang *et al.,* 2006).

A systems approach requires a good understanding and knowledge of the pest's biology as well as specific pest host interactions (Jang & Moffet, 1994). Such a systems approach can therefore only be utilised if new evidence regarding host status of *B. invadens* is available such as host prevalence, non-host status of a specific cultivar, host status regarding susceptibility for oviposition at or after a specific ripening stage or, host plant defence mechanisms being efficient to prevent oviposition or egg development.

Phytosanitary measures implemented in a systems approach are either dependent on each other to achieve an appropriate level of protection or they may be able to act independently of each other (FAO/IAEA, 2011).

The PRA results in chapter 3 identified the levels of risks which should form the baseline for the NPPOZA to determine management measures applied in a systems approach. Such a systems approach should be managed by the exporting country (Jang *et al.,* 2006).

4.3.1.4 Recommended measures for a systems approach.

Pest surveillance forms the keystone of all management decisions during a systems approach (Quinlan & Ikin, 2009). This should include fruit cutting surveys. In a systems approach several phytosanitary measures can be combined to reduce the risk of the pest to an acceptable level. These phytosanitary measures could be used as a combination of measures which individually cannot provide a satisfactory result on its own and are therefore dependant on each other to lower the risk. Alternatively at least two independent measures can also be used or measures that on their own have a significant or desired effect (FAO, 2009).

The surveillance results of a specific area from where fruit is destined to be exported from should determine the independent measures in the systems approach. Examples where surveillance is critical are when exports are allowed from a pest free area or an area of low pest prevalence (FAO/IAEA, 2011). The NPPOZA should require surveillance records from its trading partners from the production areas of each host commodity destined for import before mitigation measures are determined (Quinlan & Ikin, 2009).

The production and pre-harvest period provides the best opportunity for proper monitoring and the determination of FTD numbers.

IPM measures may form part of the normal production cycle and need to be encouraged as far as possible (Jang *et al.,* 2006). IPM may already include measures such as sanitation, bagging of fruit, male annihilation, removal of alternative hosts or reservoir hosts in the area, protein bait sprays, the application of bait stations, mass trapping, the application of the sterile insect technique (SIT) or the release of biological control agents which could act as dependant measures (FAO/IAEA, 2011; Jang *et al.,* 2006). Orchard sanitation programs need to be conducted weekly. Workers must collect dropped or none harvested fruit on a daily basis in such a way that the whole orchard is cleared in seven days. Collected fruit need to be disposed off. This is to ensure that *B. invadens* cannot complete its life cycle through infested fruit emanating from the resident orchards. Care should be taken to conduct the sanitation in such a way that the sanitation process has the least impact on parasitoids.

Timing of fruit harvesting may assist with the reduction of infested fruit of certain cultivars as the fruit may not be suitable for oviposition yet or is not yet recognised by female fruit flies as hosts (FAO/IAEA, 2011). Fruit hosts such as some avocado cultivars may not be a good fruit fly host and is only susceptible to oviposition after the fruit has been harvested (Armstrong, 1991). In the case of Sharvall Avocados exported from Hawaii to California a systems approach against *B. dorsalis* was developed (Follet *&* Vargas, 2010). A similar approach could be developed for *B. invadens* once the specific susceptible fruit maturity phase is known. This may indicate that avocado is a conditional non host of *B. invadens*. The determination of host status of many fruit cultivars for *B. invadens* needs to be concluded with field collection together with field cage tests (Aluja & Mangan, 2008; De Graaf, 2010), which could act as an independent management measure (FAO/IAEA, 2011).

Dependent measures such as host fruit inspection is not a measure as such, but should be a prerequisite to verify other measures implemented before the importation of host material for *B. invadens* is allowed (FAO/IAEA, 2011). Inspection forms an important checking mechanism for compliance purposes which can be implemented during fruit set, just before harvest and after harvest (before and after packing). It is, however, labour intensive and good statistical models need to be followed for sampling in terms of the consignment size (FAO/IAEA, 2011; FAO, 2009).

Several published post-harvest treatments exist for other fruit fly species that could be applied for different fruit fly species on different host types to be used in a systems approach or as a standalone measure.

4.3.1.5 Post-harvest measures.

These measures are applied after harvest with the objective to reach the acquired level of protection with the treatment of a consignment. Post harvest treatments may be cold or hot treatment of fruit, fumigation or irradiation. When used as a standalone measure, post harvest treatments should require a high level of certainty that they will effectively mitigate the risk of fruit fly larvae to survive in fruit hosts. It is commonly called probit 9 treatments. The level of efficacy of probit 9 treatments is 99.9968% and can be applied to induce mortality, sterility or the prevention of maturity of fruit fly larvae (Follett & Neven, 2006). Probit 9 treatments dramatically reduce the infestation level of fruit flies in fruit but it does not provide complete protection, especially when infestation levels are high.

Probit 9 treatments is a standard, generally used to mitigate post harvest treatments of fruit flies and other internal fruit boring insects (Bartlett, 1996). If applied correctly probit 9 treatments will usually be sufficient to reach the acquired level of protection of the importing country. It is generally the most accepted method to apply to most countries. Before a treatment is accepted at probit 9 level a minimum of 93 613 insects must be treated with no survivors, at a confidence level of 95% (Follett & Neven, 2006).

Post harvest treatments used in a systems approach can be an independent measure on host material from an area of low pest prevalence or when a poor host is treated (FAO/IAEA, 2011). Traditionally, importing countries have relied extensively on probit 9 level for post harvest treatments of fruit fly pests (Follett & Neven, 2006).

The application of probit 9 as a single or stand alone phytosanitary measure may, however, not be sufficient if the population levels of *B. invadens* in an area cause high volumes of fruit to be infested with larvae and should then rather be included in a systems approach.

Extensive temperature tolerance tests have been completed for *B. invadens* in Austria which indicated that the same post harvest cold sterilisation as for *Ceratitis capitata* could be followed for Citrus and pome fruits (Hallman *et al.,* 2011). Similar fruit post harvest tests have been conducted in Kenya to determine cold sterilisation schedules on Citrus and Avocados. These treatment studies have been done in vitro as well as in vivo (Grout *et al.,* 2011; Hallman *et al.,* 2011). *In vitro* studies in Austria have been conducted by comparing heat and cold tolerances of *B. invadens* with *A. ludens*, *B. dorsalis*, and *C. capitata*. The cold treatment schedules which were effective against *A. ludens*, *B. dorsalis* and *C. capitata* were also effective against *B. invadens* (Hallman *et al.,* 2011).

Cold treatment schedules have already been developed and implemented for many crops against *C. capitata* by several trading partners. Furthermore, with studies conducted in Kenya, it was found that oranges can effectively be treated against *B. invadens*. Orange fruit should be cold treated at a fruit pulp temperature which is maintained at 0.9°C (±0.5°C) or lower for 16 consecutive days (Grout *et al.,* 2011). Existing cold treatments against *C. capitata* should be effective to control *B. invadens*.

No heat treatment schedule has been developed yet for the treatment of hosts such as mangoes and papaya although the tolerance tests conducted in Vienna indicated that *B. invadens* is less tolerant to heat treatment than *C. capitata*. However, more variation in heat treatment tests can be expected than the cold treatment tests and further studies need to be conducted. Therefore no heat treatment schedule can be implemented at this stage (Hallman *et al.,* 2011).

Irradiation for fruit hosts such as mangoes has been developed for Tephritidae species and could effectively be used for disinfestations of *B. invadens*. Generic dosages for fruit flies are described in ISPM No. 18 (2003), *Guidelines for the use of irradiation as a phytosanitary measure*. A dosage of 50-250Gy is recommended to prevent adult emergence from 3rd instar larvae. However, the application of irradiation requires the building of expensive irradiation plants with several additional quality checks and protocols to be followed to ensure an effective treatment and to ensure optimum dosages for each cultivar(Follett & Neven, 2006; FAO, 2009; Ogaugwu *et al.,* 2012).

It is recommended that cold treatment schedules suitable for the treatment of fruit for *C. capitata* be used for the treatment of *B. invadens*. These schedules may be utilised for specific cases such as the treatment on citrus. Post harvest treatment should be done in the exporting country or provided in transit so that the treatment period is reached before arrival. Post harvest treatments should not be used as a standalone treatment but can be used as an independent measure in a systems approach. Care should be taken to ensure that no re-infestation occurs during or after cold treatment in the exporting country. A sufficiently traceable, orchard or producer registration system must be implemented in the country of export before importation can be allowed.

4.3.2 Pathway 2: Plants imported from African countries with soil or growing material, including pot plants.

Soil or potting mixtures can originate from areas where infested fruit had been dropped and may contain pupae. The NPPOZA does allow small quantities of pot plants with their growing medium from neighbouring countries.

B. invadens occurs in Namibia, Botswana and Mozambique, which increases the risk that soil or growing medium contains viable pupae.

It is recommended that importation of plants with soil, humus, or other potting mixtures from countries where *B. invadens* occur be prohibited.

4.3.3 Pathway 3: Fruit carried by passengers or as cabin luggage in commercial vehicles and small quantities for informal trade.

The origin, production or treatment of fruit carried informally is unknown and no fruit can be allowed to enter South Africa from infested countries by people for own consumption or informal trade.

Regular spot checks should be implemented at border posts and an on the spot fine system needs to be developed which interacts with an awareness campaign to ensure maximum cooperation from the public. An on the spot fine system can also be implemented with regard to quarantine areas within South Africa.

However, an on the spot fine system is not possible under the current legislation, the Agricultural Pests Act and R110. The current legislation provides for sentencing that could be either a fine or jail time. This entails that an offender must first be found guilty in a court of law.

An on-the-spot fine system provides for an immediate admission of guilt system which would allow an offender to pay a fine immediately to the relevant executive officer. Such a system should be implemented especially in the case of offenders moving host material across the border without permits and would include passengers transporting host material for own use. In such cases an on the spot fine system can be an effective disincentive.

It is recommended that the Agricultural Pests Act be amended to ensure a more flexible control system both for import and national regulation. Existing legislation (Agricultural Pests

Act) enables the national control of *B. invadens* but may have to be amended to optimise control measures such as the inclusion of an on the spot fine system. This should be supported by visible awareness programs regarding the movement of fruit from an infested area to a non infested area or within an infested area in South Africa. Additional support would be required at the Beitbridge border post such as the implementation of sniffer dogs and x-ray machines to detect fruit in baggage. It is important that different organs of state work together to prevent the introduction of fruit as part of cabin luggage in vehicles. This would require new role player involvement other than those indicated in Fig. 4.1.

4.3.4 Degree of uncertainty.

The application of any measure has a degree of uncertainty to the extent that it was implemented as indicated in ISPM No11. This uncertainty causes a risk factor which may be involved during the implementation of phytosanitary measures or the measures itself due to lack of knowledge and data, or to natural variability. This should be considered in the pathway initiated PRA and an extrapolation from other known situations or PRAs should be taken into consideration to create additional assurance. The degree of uncertainty should be determined and documented. A pathway initiated pest risk analysis would be able to identify the degree of uncertainty for the host plants destined to be imported into South Africa. The degree of uncertainty will differ between host plants, phytosanitary measures implemented by exporting countries, climatic zones, the level of infestation or population levels in a particular area, production control and packing and traceability methods used. NPPOZA who is the importing country needs to obtain additional information and assurances from the exporting NPPO regarding these uncertainties (FAO/IAEA, 2011; FAO, 2009).

The lack of permanent surveillance programs in exporting countries leads to uncertainties in the management of *B. invadens* as the efficacy of phytosanitary measures applied in the pre-harvest phase is dependent on fruit fly population levels.

The successful planning and implementation of control measures to lower fruit fly population and fruit infestation levels such as orchard sanitation, fruit bagging, the male annihilation technique, bait application technique and sterile insect technique which is highly dependent on surveillance results as an auditing and monitoring action is therefore influenced.

Uncertainties regarding the option to accept a declaration of pest free area, pest free place or site of production and an area of low pest prevalence increases with unreliable surveillance records.

Lack of knowledge causes uncertainties regarding the harvesting methods and time and fruit maturity in a diverse agricultural production area. Uncertainties exist regarding the efficacy of post harvest treatments and its application in terms of the standard of equipment and facilities as well as the consistency of treatments over time, especially cold treatments which occurs over several weeks.

4.3.5 Conclusions.

Uncertainties from the importing country regarding the processes followed in the exporting country can be resolved by the importing country having access to additional information.

NPPOZA could address this through continuous bilateral engagement with potential trading partners to emphasise the importance of surveillance records to ensure safe trade. Additional information required would include surveillance data which starts before and during a pre-harvest treatment period with supportive treatment records. It continues with surveillance data and post-harvest treatment records such as temperature data over the duration of treatment application and is confirmed by evaluating official inspection reports (FAO/IAEA, 2011).

Additional assurances should be included in the import requirements such as harvesting methods, transport methods from the production site to the packing facility and from the packing facility to the shipping facility to avoid post-harvest oviposition on fruit. These can be utilised effectively as a measure in a systems approach.

Traceability of consignments is important for follow up of any uncertainties or non-compliance. This can be achieved by implementing a registration system of all producers with a production unit code (PUC) which is indicated on the packaging.

Pre–clearance inspections allow the importing country to obtain additional assurances regarding the integrity of the whole process, especially with a systems approach. These inspections are conducted by the importing NPPO in the territory of the exporting NPPO and can be conducted at any point in the process (FAO/IAEA, 2011).

Phytosanitary certification is an official certification system which provides assurances to the importing country in the form of a declaration (or declarations) of implementation of several measures or with regard to the status of the pest in an area (FAO, 2009).

As an example, an import condition for the importation of citrus to South Africa from an infested country should require an additional declaration on the phytosanitary certificate that:

the fruit is harvested from a pest free production area

or

the fruit is harvested in a pest free place of production surrounded by an area of low pest prevalence which will act as a buffer zone

or

the fruit is harvested from an area of low pest prevalence and cold treatment was conducted at 0.9 ^{o}C pulp temperature for 16 consecutive days without interruptions (Grout, *et al.,* 2011).

The exporting phytosanitary authority must declare on the phytosanitary certificate that the consignment of host material in question complies with the requirements of the importing country. They must also provide assurances such as traceability and preclearance. The import conditions and subsequently the additional declaration on the phytosanitary certificate may differ as it is dependent on the host commodity, the country of production and the type of phytosanitary measures which were agreed upon between trading partners. Care should be taken that the measures applied are equivalent to ensure the acquired level of protection is achieved (FAO, 2009).

4.4 Phytosanitary measures for national control.

4.4.1 Introduction.

The detection survey revealed that *B. invadens* did occur in the study area. The source of the introduction is not known and re-incursions are a probability. Permanent exotic fruit fly detection surveys are essential to ensure early detection of fruit flies such as *B. invadens* and should continue. This may have to be increased and optimised in terms of the areas it have already been detected before. National legislation exists as indicated in section 4.2 and the Agricultural Pests Act as well as the control measures R110 have to be amended as recommended in 4.2.3 to include the possible implementation of a notification system for the declaration of quarantine areas.

Control measures are affected by processes such as biological, ecological, technical, economic, trade (local and international), and social and political processes and follow a hierarchy of systems (Norton & Mumford, 1998).

A corrective action plan is therefore necessary to address new incursions.

4.4.2 Corrective and quarantine actions.

Corrective actions must be implemented when the status of a pest free area has been compromised or is in danger to be compromised. The territory of South Africa and therefore also the Musina area is seen as a pest free area and a corrective action plan should be drafted and regularly updated according to ISPM No.26. Local control measures should follow an approach with the initial goal to eradicate the pest from predefined areas.

The NPPOZA should ensure that a corrective action plan is in place which provides details regarding the phytosanitary measures implemented. These measures should be developed in cooperation and through a consultation process with fruit fly experts, fruit industry members and local producers. The aim of the corrective actions is to reinstate the pest free status of the pest in the area. This may include phytosanitary measures to continuously lower the pest population in an area until it is no longer sustainable and the pest is eradicated from the area.

The risk of this pest to spread to other areas in South Africa is high and the current approach to eradicate *B. invadens* after the second fruit fly specimen is detected should be continued. Eradication actions should continue even though the surveillance results from 2010 and 2011 indicate repetitive introductions, which may continue in future (Liebhold & Tobin, 2008). Care should be taken to optimise the local control measures so that it has the least environmental and economic consequences to the area and are the least restrictive to local producers.

The Musina area borders a part of Botswana and Zimbabwe with the Limpopo River. Large parts of the area are not under crop production but consist of natural reserves or game and cattle farms. Eradication should include actions which involve the simultaneous application of area wide control with the implementation of the male annihilation technique and the bait application technique as the current action plan dictates.

Area wide control methods should however not be used as a single approach in an effort to eradicate this pest but should be combined with several other production control methods to lower the pest population in an area (Hancock *et al*., 1998). Surveillance should continue throughout the eradication phase to determine the FTD values. Before eradication can be established, a period of at least one life cycle should be allowed without any male annihilation methods applied to determine the area as free from the pest.

The measures used to lower the pest population for eradication should aim to control fruit flies at every life stage (Norton & Mumford, 1998). Fig. 4.3 illustrates a typical fruit fly control flow chart, which is optimised for the control of *B. invadens*. At each interaction point with the fruit fly life stage the relationship impact is indicated with a "+" for positive impact and a "–" for negative impact, respectively. Interactive relationship impacts are also indicated such as the impact of fruit bagging on parasitoids. The direction of the flow is indicated with an arrow (Norton & Mumford, 1998).

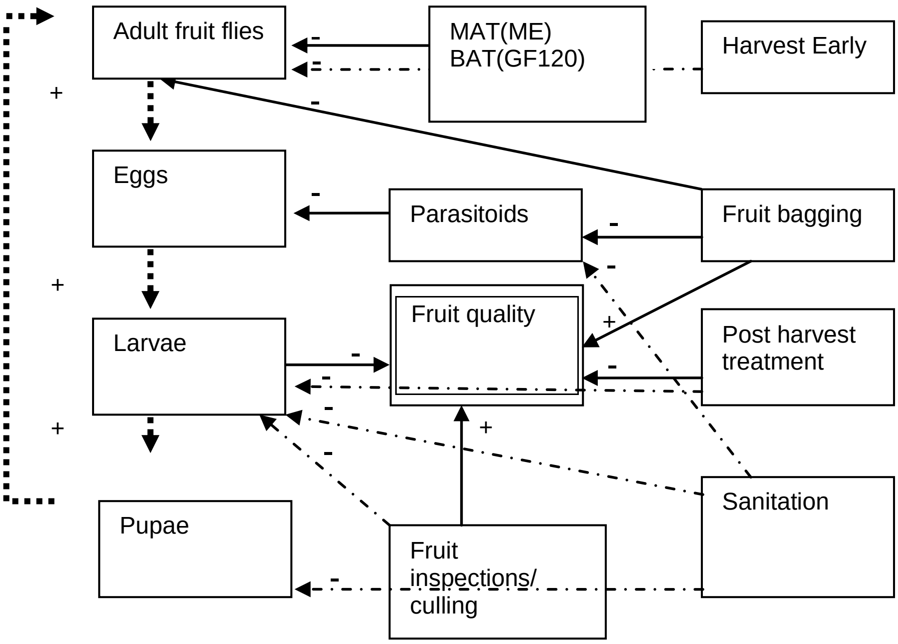

Figure: 4.3. A flow chart with qualitative relationships between fruit quality, fruit flies and fruit fly control as adapted for *Bactrocera invadens*. Control using the male annihilation technique (MAT) using Methyl Eugenol (ME) as a parapheromone and the bait application technique (BAT) using GF120 as protein bait spray is indicated as an adaptation for *B. invadens* to be used in combination with other cultural control methods such as fruit bagging and biological control such as parasitoids (After Norton & Mumford, 1998).

The aim of the corrective actions implemented in the study area in 2010 was to eradicate the area from a possible founder fruit fly population. The measures implemented focussed on individual land owners which were placed under quarantine and subjected to phytosanitary conditions applied on their individual production areas. These phytosanitary conditions included surveillance, compulsory orchard or field sanitation, inspection and fruit cutting as additional measures to the area wide control, indicated in the existing action plan (Manrakhan *et al.,* 2009). Movement of host material was only allowed with a permit subjected to compliance to these conditions. Conditions for movement control, post-harvest treatments and waste management were implemented. Several of the measures implemented formed part of quarantine actions.

Corrective measures differ from quarantine actions where the latter focuses on confinement of a potential contaminant (infested fruit from spreading) while corrective measures aim to bring the potential existing population density down to an acceptable level (Follet & Neven, 2006, FAO, 2011). Typical quarantine actions would be to secure a moving citrus consignment with insect proof netting on its way to a juicing factory. These measures are extended to be implementable at the factory to prevent the potential further development of larvae in fruit especially in factory waste products.

Corrective measures and quarantine actions cannot be separated in practice and should be applied together to enhance the chances to control the pest in the country when the aim is to eradicate the pest from an infested area as illustrated in Fig. 4.4.

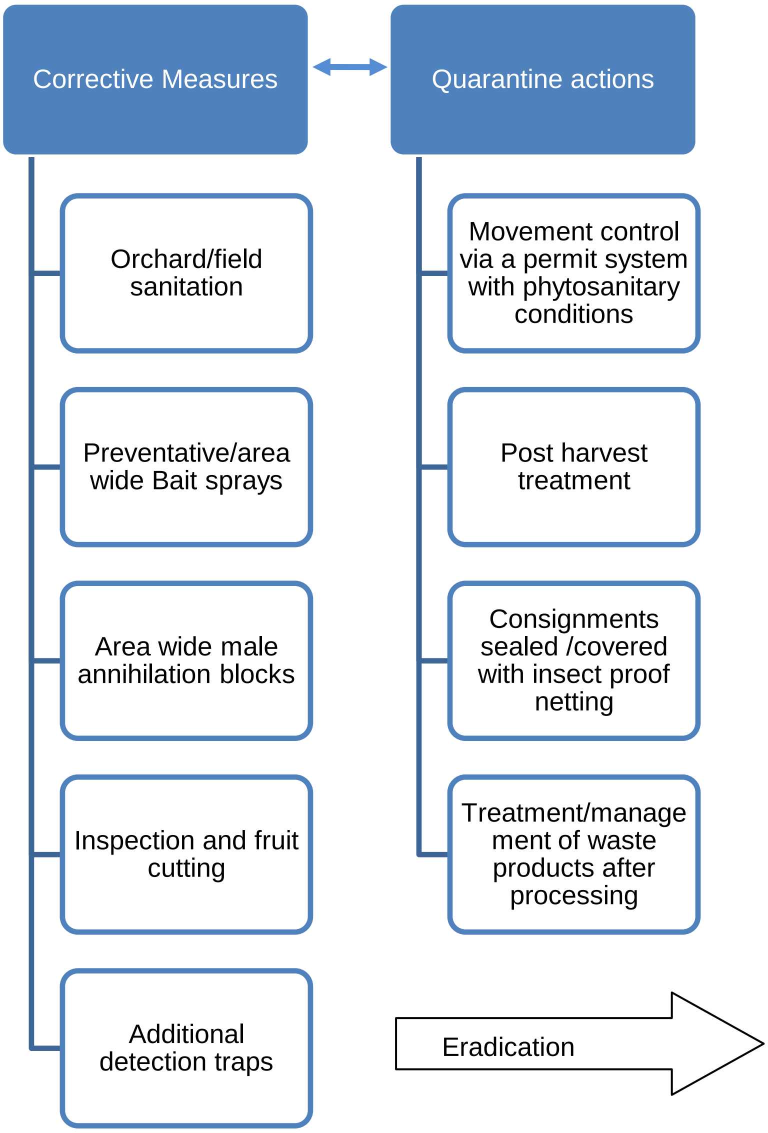

Figure: 4.4 Corrective and quarantine actions to jointly achieve eradication in the affected area. The bait application technique (BAT) and Male annihilation technique (MAT) are both area wide control methods which should be applied to eradicate *B. invadens* in an area.

4.4.2.1 Surveillance.

During the eradication program in 2010 in the Musina area, additional traps were placed to augment the existing trap grid which formed part of the national survey or the delimiting survey traps in the quarantined area. This was done, either by producers themselves, industry or the NPPOZA and maintained and serviced during eradication (Manrakhan *et al.,* 2011).

Areas that normally do not receive any fruit fly treatments such as house gardens with host plants and neglected orchards should also be subjected to additional surveillance trappings. It is important to note that for eradication purposes, the spread and dispersal of the pest within the affected area as well as out of the affected area should be controlled. As soon as fruit is collected and moved around the area it increases the risks of the pest spreading and therefore additional surveillance is needed. Both male specific and male inclusive fruit fly lures should be used in these areas which would be ME and Biolure as used during the detection survey as indicated in paragraph 2.2.2.2.

Traps should be placed at all fruit sorting, treatment, sanitation and disposal areas, animal feeding areas, or composting sites, pack houses, produce reception areas, such as fresh produce markets or ports of exit. Trap placement should be at least at a ratio of two ME traps per km^2 and one to two Biolure traps per km^2 which would normally only be the first square km due to the small size of these areas.

House gardens in the study area were generally not treated in the same manner as production areas with regard to specific fruit fly control measures. These gardens are however well watered and may provide shelter for fruit flies as well as sustaining alternative host plants. Additional traps should be placed in house gardens in an affected area at the same ration as in production areas for detection and should include both ME and Biolure traps.

4.4.2.2 Orchard sanitation.

Orchard or field sanitation forms an important cultural control measure to control fruit flies such as *B. dorsalis* in mango orchards (Verghese *et al*., 2004).

Sanitation was done weekly during the 2010 eradication of *B. invadens* in the affected area (Manrakhan *et al.,* 2011). As the targeted fruit fly was never detected in fruit, the sanitation included all house gardens, fields and orchards in the affected area. The removal of fruit was done on a daily rotational basis by scouts and workers throughout the orchards over a week's time and repeated every week. Fruit targeted in the sanitation program did not only focus on dropped fruit in orchards, but was extended to include the removal of fruit left over after harvesting from trees. Fruit and wet waste rejected or, dropped at reception, processing sorting, packing and shipping points throughout the distribution chain, were also collected and included in the sanitation program. Most of the fruit was disposed of by burying it in a hole covered with 50cm of soil. Compost from the previous season was heaped on top of the soil and covered in the same manner (Klungness, *et al*., 2005). Fruit buried in the soil or in compost heaps stayed covered for at least five weeks.

Alternatively some of the producers shredded the fruit with mechanical shredders and left it to dry in the sun and others fed it to game at allocated game feeding areas.

The destructive process used in 2010 for sanitation such as burying is useful for eradication processes but can have a negative effect on possible parasitoids which parasitize eggs and larvae in the fruit (Manrakhan *et al.,* 2011).

It would therefore be useful to encourage parasitoid development for the biological control of *B. invadens*. No naturally occurring parasitoid for this fruit fly has been identified in South Africa yet. A bioassay study was done by Mohamed et al, (2010) to test the suitability of *Fopius arisanus* (Sonan) (Hymenoptera: Braconindae) as an egg parasitoid for *B. invadens.* The host preference, host acceptability for oviposition and physiological suitability of *B. invadens* and five other indigenous tephritid fruit fly species in Kenya namely, *C. capitata* , *C. cosyra*, *C. rosa*, *C. fasciventris* and *C. anonae* Graham were compared. *F. arisanus* originates from Asia and could be collected in Sri Lanka where *B. invadens* also occurs. Furthermore, it is also a successful parasitoid that is bred in captivity for augmentative release to control *B. dorsalis* in Hawaii. However, it is not indigenous in Africa but was subsequently introduced into Kenya. The effectiveness of indigenous parasitoids was also compared (Mohamed *et al.,* 2010). *Psyttalia cosyrae* (Wilkinson)(Hymenoptera: Braconidae) and *P. phaeostigma* (Wilkinson) two solitary parisitoids and one gregarious parisitoid *Tetrastichus giffardii* Silvestri (Hymenoptera: Eulophidae) were tested and although *B. invadens* was readily accepted by *T. giffardii* and to a lesser extent by *P. cosyrae* and *P. phaeostigma,* nearly all eggs were encapsulated in the larvae of *B. invadens* which rendered these parasitoids unsuitable for biological control. Of all the host fruit sampled in East and West Africa (over 34 ton) no parasitoid emergence has so far been documented. It was established in the study that *F. arisanus* has a strong preference for *B. invadens* which resulted in 73,6% parasitism. For *C. anonae, C. capitata*, *C. cosyra*. *C. fasciventris* and *C. rosa* a much lower rate of parasitism of 8.23, 36.41, 27.28, 0.0 and 1.58, respectively occurred (Copeland *et al*., 2006; Mohamed *et al.,* 2010). *F. arisanus* has not been released or detected in South Africa according to the Biosystematics division of the Agricultural Research Council, Plant Protection Research Institute, Pretoria.

Augmentariums, a type of field applied insectariums, can be constructed in a simple way by covering the areas where fruit is collected with an insect net which have holes too small for adult fruit flies to escape but big enough to allow third instar parasitized larvae to move through it and to allow parasitoids to escape (Klungness *et al*., 2005). The nets should be placed on the soil with the edges buried 30cm deep to secure the nets. Fruit is spread evenly on top of the nets. The fruit should not be spread closer than 70cm from the edges. Fruit fly larvae do not generally borrow deeper than 8cm (Klungness *et al*., 2005). This allows for parasitized third instar larvae to emerge from the fruit and to move through the net into the soil to pupate. The adult parasitiods emerge from the pupae and are small enough to escape through the net. Unparasitized larvae will develop normally and adult fruit flies can emerge from the pupae through the soil but are too big to escape through the net and will die. This method therefore allows for adequate parasitism and efficiently destroys all un-parasitized specimens. This method however does pose a risk as there may be pupation on the nets underneath the heaped fruit with subsequent adult emergence of adult fruit flies. This will depend on how the fruit is spread on the nets (Klungness *et al*., 2005).

It is recommended that rigid sanitation practices are followed to lower population levels in the areas to ensure eradication. Fruit should be buried with 50cm of topsoil for sanitation during an eradication program. This can however be optimised to ensure minimum impact on possible parasitoid development (Pinero *et al*., 2009). Parasitoids such as *F. arisanus* may be released as part of an augmentative biological control program and it would be beneficial to ensure as much possible emergence of *F. arisanus* from orchard sanitation activities. Parasitoids can be considered as having an added impact on fruit fly populations even though there may still be adults emerging from fruit heaps on the nets (Klungness *et al*., 2005).

4.4.2.3 Fruit inspection

Regular fruit inspection and cutting were done at production units, processing plants, pack houses, as well as any other sorting and grading points in the quarantine area. It was also done where fruit from the quarantine area is received, sorted, processed, handled or sold outside the area during 2010 (Manrakhan *et al.,* 2011). Fruit in orchards and discarded in rejection bins were also inspected. Only one inspection per consignment or lot or truckload was conducted. Members of the NPPOZA operational side (DAFF inspectors) visited the area on a weekly basis and inspected fruit at different production units at different time intervals. Fruit could only be moved to processing plants after inspection in terms of the control measures applied.

The time and point of inspection is not important as long as the consignment is isolated in such a way that it excludes re-infestations of host material while the consignment is moved from one point to another through or within the affected area (Cantrell *et al*., 2002).

These inspections should not coincide with or replace the normal quality inspections as it is an additional inspection for the most likely pathways at the most likely time to detect *B. invadens*.

Inspections at any particular site must be planned together with producers or pack house managers, shipping agents and port managers. As *B. invadens* is an internal feeder, fruit cutting should form part of the inspection. Fruit cutting should be done on rejected fruit after sorting in storage containers such as bulk bins, at ambient temperature. Infested fruit should be placed into rearing cages after inspection. Fruit fly larvae detected during the cutting process must also be sent for molecular taxonomic analysis. Adult fruit flies from rearing cages should be identified by a recognised fruit fly taxonomist.

If fruit fly infested fruit is found during quality inspections it must be put into rearing cages. Any other rejections based on fruit fly oviposition marks, or larvae present, must also be put into cages.

4.4.2.4 Post Harvest Treatments.

Post harvest measures can be implemented once an increase in population levels is noticed with detection of larvae in fruit. This should be conducted when fruit is moved out of the affected area to be sold on the national market regardless if it is through a formal marketing system or for the informal trade. The Pest Risk Assessment determined that the informal marketing of host material poses a high risk of the fruit fly to establish in new areas.

The implementation of such treatments would be difficult as there are currently no post harvest treatment facilities in the Musina area or in most production areas in South Africa. Post harvest measures should be on probit 9 levels to provide adequate protection (Follett & Neven, 2006; FAO/IAEA, 2011). Cultivar specific Probit 9 treatments have not yet been developed to be used on fruit for the post harvest treatment of *B. invadens* for the majority of host commodities.

Treatments must be done before fruit is moved out of the quarantine area. These treatments can be used on fruit sold to small scale traders who collect unpacked fruit or on farm/ pack house sales in cases where there is no grading process. The same must apply for fruit does not qualify for sales to fresh produce markets after grading. Care should be taken to ensure that re infestation does not take place after treatment. Post harvest treatments could also be done at treatment facilities close to, or at fresh produce markets of pack houses outside the quarantine area. Post harvest treatments for national control have not yet been developed for all the host commodities of *B. invadens* and should be similar as which are recommended for importation of fruit from *B. invadens* infested countries (FAO, 2009).

4.4.2.5. Area wide Fruit Fly control.

The immediate application of area wide control measures is essential to eradicate new introductions of pest fruit flies from an area (Cantrell *et al*., 2002; Hancock *et al*., 1998). A joint approach should be used by using both the male annihilation technique and the bait application technique (Vargas *et al*., 2010).

Methyl Eugenol is an effective attractant for *B. invadens* males to be used together with an insecticide such as malathion for male annihilation in a specified area. Spinosad (Spinosyn A, D) mixed with protein bait such as GF120 Naturalite (GF1111) provides effective control of *B. invadens* (Vayssières *et al*., 2009) as it attracts both males and females.

The area from Pontdrift to Beitbridge border posts (Fig 2.2) was quarantined after the detection of male fruit fly specimens in 2010. This included areas which were not cultivated such as areas of natural vegetation, unused or barren fields and areas cultivated with non-host crops such as cotton and maize (Manrakhan *et al.,* 2011).

MAT fibre board blocks (Invader-b-Lok) were placed in the area at 40 blocks per km^2 covering mostly host production areas but also areas alongside the Limpopo river on the border fence. The MAT blocks were effective for eight weeks and over 12000 blocks were used in the area (Manrakhan *et al*., 2011).

The BAT technique was applied using 4-7 day protein bait sprays (GF120 Naturalite, DOW AgroSciences Southern Africa (Pty) Ltd) as ground–based sprays for eight weeks on host material at production sites. The GF-120 was applied over an area of $25km^2$ on citrus and cucurbit crops. It included 19 production sites within the quarantine area. Two aerial protein sprays (Malathion ULV 1130g/L, Avima (Pty) Ltd) mixed with a protein hydrolysate, LokLure (Tsunami Plant Protection (Pty) Ltd, South Africa) sprays were applied alongside the

Limpopo river between Pontdrift and Beitbridge as well as on citrus orchards. The area was declared free from *B. invadens* after no specimens were detected in the delimiting traps for four weeks after the MAT blocks were removed from the area (Manrakhan *et al.,* 2011).

It is recommended that the area wide application of BAT by using GF-120 should be continued for eradication purposes in cases of future introductions of *B. invadens* to South Africa. It can be applied either as ground sprays or as aerial sprays with eight consecutive weekly sprays in a quarantine area which must be followed up with a 4-8 week monitoring period.

The application of malathion protein hydrolysate mixtures should however be discouraged as it has an 18 day withholding period and although malathion (ULV) is highly biodegradable it still has an adverse effect on bees and is very toxic to fish on contact.

However, the application of GF-120 is up to 4 times more expensive than malathion (ULV) /protein hydrolysate mixtures and may have a negative impact on production costs in industries such as tomatoes, peppers and bananas which did not previously use agricultural chemicals to control fruit flies. GF120 has been used for indigenous fruit fly control on produce destined for export by citrus, avocado and mango growers due to the low risk of pesticide residue rejection in comparison with malathion based products.

Care should be taken to ensure that house gardens and neglected orchards or host trees are also treated in the same way as production units within the affected area.

4.4.2.6. Fruit movement.

Fruit movement inside, through, as well as out of a quarantine area should be safeguarded (Cantrell *et al*., 2002). No fruit produced in the area was allowed to be moved outside the quarantine area without a permit issued by the NPPOZA according to R110 quarantine action. Fruit that was moved from the affected area in 2010, to a processing or handling point outside the area after the detection in 2010 would have caused the destination point to be considered as a quarantine area and any other associated produce handled at the same facility would also have been at risk (Manrakhan *et al.,* 2011).

Trucks and/or cargo were sealed in such a way that fruit could not fall from the truck during transport. Trucks were sealed and covered in such a way that no adult fruit fly which may have entered the pack house and consignment during packing could escape during transport.

It is recommended that the transport of packed fruit through or from a quarantine area should be secured on pallets and loaded in a container which is sealed after inspection by NPPOZA. Equivalent to that would be to transport host material in sealed refrigeration trucks. This should be used especially for high quality and high value consignments destined for the export market and pre-packed vegetables to local markets (Cantrell *et al*., 2002). Transporting fruit in sealed conveyances ensures that no oviposition takes place during transport. It also ensures that possible infested fruit cannot fall off trucks during transport or is removed by humans or baboons. This mode of transport would not always be possible for all types of fruit as it is costly, needs to be prearranged long in advance and conveyances of this type are not always available. Based on the type of movement, destination and whether or not a post harvest treatment has been conducted already, the type of conveyance could also differ.

4.4.3 Interaction of management measures.

The hierarchy of systems identified or followed by the NPPOZA to control *B. invadens* as indicated in paragraph 4.1 should be expanded to include more role players and the current action plan may have to be amended subsequently. The backbone of the current action plan is the ongoing surveillance actions where all the major fruit industries participate for cooperative participation (Manrakhan *et al*., 2009). The purpose of the surveillance is to enable early detection of the fruit fly so that the cost of eradication can be minimized (Mumford, 2005). Surveillance should therefore be expanded to ensure early detection (Liebhold & Tobin, 2008) for more feasible eradication.

The good relations with fruit industry bodies should continue and extent to local producers, provincial government as well as local government. Local communities should be engaged on a regular basis by local government officials such as extension officers to ensure the implementation of management measures on community level (Ndiaye *et al.,* 2007; Kwasi, 2008). This is especially important with the application of local control such as the setting up of road blocks, implementation of fines and extension and awareness services regarding the control measures for *B. invadens*. Interactive relations also need to be extended to other fruit industry bodies or representative organisations to include representation of host commodities such as papaya, bananas, guavas, tomatoes, peppers and cucurbits. As soon as new detections are reported these new fruit fly pest incursions should be contained in the areas detected (Cantrell *et al*., 2002). This is only possible with a good interactive plan and cooperation of all possible role players.

The current action plan (Manrakhan *et al*., 2009) requires a quarantine area of 5km radius surrounding the first positive detection to be implemented with immediate application of phytosanitary measures which regulates the movement of fruit out of an affected area. This can be achieved with the assistance of local government way of setting up roadblocks and road signs after these detections (Hancock *et al*., 1998). With new detections producers need to be encouraged to cooperate with the NPPOZA to implemented management measures. This may require additional incentives to the producers who are cooperating.

The fruit industry bodies play a vital role in the implementation of management measures. They interact with their respective members and encourage farmers to produce for specific markets which are already adhering to specific IPM and control measures which are in line with phytosanitary regulations of importing countries and quality standards. This can further be supported by industry extension officers and study group leaders from the various organisations, local and provincial government as well as pesticide representatives. Compulsory surveillance for *B. invadens* should be included as a prerequisite for registration to export markets. This will increase the existing surveillance network tremendously and will provide a bigger confidence level to importing countries as well as NPPOZA regarding the presence or distribution of the fruit fly in specific areas within South Africa. The existing industry forums within the NPPOZA should be continued and action groups expanded to set the platform for expert participation.

4.4.4 Interaction matrix for *B. invadens.*

An interaction matrix has been compiled in Fig. 4.5 to describe different factors in an agro-ecosystem and their interactions which may have an influence on the control of *B. invadens*. The purpose of the matrix is to identify major components in the possible management system for *B. invadens* especially when it has been introduced in the country such as the study area. It also aims to identify the impact of management variables on the system. The matrix can be used to identify relationships between different factors influencing the identification of research needs (Norton & Mumford, 1994). The SABIFF action plan took into consideration data on *B. invadens* which was available at the time, such as certain life history data. This included data on development time, host ranges, distribution areas, pheromone and attractant data, trap data and food mediums for production purposes (Manrakhan *et al*., 2009; 2011). This was used as a reference to populate the matrix in terms of physical factors and area wide control. Major relationships and the various interactions are indicated. The matrix is filled with dots, open dots and question marks which indicates direct or major (●), indirect (o) or minor interaction or a possible research need (?) respectively. A blank indicates no conceived interaction.

4.4.4.1 Matrix components and interactions.

The major component categories used were climate, fruit fly management measures, crop factors, other fruit fly species and natural enemies as well as pest factors. Interactions between climatic factors in the region, existing regional indigenous fruit flies and possible existing exotic fruit flies, crop factors such as cultivar, fruit period and harvesting time were used. Management measures applied in the area included cultural control methods as well as chemical and area wide control measures and post harvest measures. Post harvest measures such as cold treatments, irradiation and fumigation were included as factors in the matrix. Biological factors for *B. invadens,* such as immigration, mating success and life cycle stages of fruit flies were used. These factors are subdivided as illustrated in Table 4.1

Indigenous *Ceratitis* spp. which may share citrus and mango hosts and *Dacus* spp. which may share cucurbit hosts with *B. invadens* in the area were used. In addition to that possible exotic fruit flies attracted to methyl eugenol were also included in the matrix. Some of the crop management measures used for control of the indigenous fruit fly species which occurs in the production area may have an impact on *B. invadens* and can be used as a phytosanitary measure in a quarantine area. Those phytosanitary measures are implementable from the production phase up to post harvest were included in the matrix to indicate the possible relationships these measures may have with other matrix factors. Area wide control included in the matrix was the male annihilation (MAT) using methyl eugenol, bait application (BAT) using GF120 and the sterile insect technique (SIT) releasing sterile *B. invadens* male specimens.

An important factor which has not yet been considered by the NPPOZA is the release of a biological control agent. The augmentative release of an effective parasitoid may benefit control actions mainly at higher pest population levels (Vargas *et al*., 2007; Mohammed *et al.,* 2010). It has already been indicated earlier that sanitation may have a negative impact on parasitoids. The matrix illustrates that there may be several other factors interacting with natural enemies and parasitoids. These include regional factors as well as other crop system factors. If post harvest treatments are used for national control it may also have an impact on egg parasitoids.

Table 4.1 Factors used as general components to determine relationships in an interactive matrix.

Climate	**Management measures**	**Crop Factors**	**Other fruit fly species and natural enemies**	***B. invadens* biological factors**
Relative humidity	Sanitation	Cultivar	*Ceratitis* spp.	Immigration
Temperature	Soil Drench	Fruit set time	*Dacus* spp.	Emigration
Altitude	Male Annihilation Methyl Eugenol (ME)	Fruit period	Other ME respondent *Bactrocera* spp.	Mating success
	Bait Application	Harvesting time	Natural Enemies and parasitoids	Sex ratio
	Sterile Insect Technique	Wild hosts		Adult density
	Cold Treatment			Adult duration
	Hot treatment			Oviposition
	Irradiation			Egg survival
	Fumigation			Larval survival
				Pupal survival

The augmentative release of a parasitoid during outbreaks may not be sustainable within ultra low pest populations as experienced in 2010 but should be considered. However, classical biological control could be considered for low populations (Waage & Hassell, 1982). The introduction of exotic natural enemies should be considered to address the ability of *B. invadens* to encapsulate indigenous parasitoids. Action steps to import and breed local populations of *F. arisanus* to be utilised as a contingency measure should be considered.

Climatic factors used include abiotic factors such as relative humidity (RH), temperature and altitude. Indigenous fruit flies, natural enemies and parasitoids will be adapted to these factors. The increase of temperature, rainfall and RH has a positive effect on *B. invadens* populations (Vayssières *et al*., 2005). The increase of *B. invadens* adults in an area can have a negative interaction with *C. cosyra* populations (Duyck *et al.*, 2004; Ekesi *et al.,* 2009).

B. invadens have a large host range including several wild hosts. The wild hosts may contribute to a seasonal population flux between the native vegetation of the dry savannas and commercial orchards (Vayssières *et al.,* 2009a). This contributes to keeping *B. invadens* populations high during periods when the commercial orchards are not producing fruit. Therefore, wild hosts must also be included in control programs (Vayssières *et al.,* 2009a.)

The interaction with different cultivars and fruit hosts in the area over a specific time is important because the availability of suitable hosts will have a major impact on the prevailing population of *B. invadens.* Cultivar differences of a specific host will also result in different times that fruit is ripening and susceptible to oviposition by different fruit fly species (Vayssières *et al.,* 2009a). Host status of each fruit type can be determined for specific cultivars and can therefore also be utilised in management programs as a factor to be used in a systems approach to manage *B. invadens.*

The matrix indicates the importance of fruit fly control using cultural methods such as sanitation which has an effect on several fruit fly species including *B. invadens*. Sanitation has a direct negative impact on eggs and larvae of fruit flies. The matrix only illustrates the relationship with *B. invadens* eggs and larvae. Although labour intensive, it is recognised as an important step in fruit fly control (Kwasi, 2008). The inclusion of soil drenching has a major effect on larvae emerging from fallen fruit without having an effect on natural enemies of other pests such as scale insects (Peña *et al.,* 1998). However, no agricultural chemical has been registered in South Africa to be used as a soil drench, that may be effective against fruit flies in terms of the Fertilizer, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947).

Major measures to eradicate *B. invadens* from an affected area are the application of MAT, BAT and the possibility of SIT. Both MAT and BAT have been used in several international fruit fly eradication programs such as the program to eradicate *Bactrocera papayae*, the Queensland fruit fly (Cantrell *et al*., 2002) and the *Bactrocera zonata* fruit fly plan (FAO/IAEA, 2000). This is supported by additional measures which may already be used in different cropping systems depending on the host plant (Kwasi, 2008). Spot bait sprays also reduce the insecticidal effect on other natural enemies (Peña *et al.,* 1998). The use of GF120 Naturalite (GF1111) with Spinosad (Spinosyn A, D) mixed with a protein bait as the active ingredients is effective for the control of *B. invadens* as a bait spray as well as other resident fruit fly species (Vayssières *et al.,* 2009b).

	Climate			MM									CF					O FF & NE/P				*B. invadens*									
	RH	Temperature	Altitude	Sanitation	Soil drench	MAT ME	BAT	SIT	Cold T	Heat T	Irradiation	Fumigation	Cultivar	Fruit set time	Fruit period	Harvest time	Wild hosts	*Ceratitis*. spp	*Dacus* .spp	ME exotics	NE/P	Immigration	Emigration	Mating	Sex ratio	Adult density	Adult duration	Oviposition	Eggs	Larvae	Pupae
Climate																															
RH		•	•			○	○	•	○	○		•	•	•	•	•	•	•	•	•	•	•	•	•	?	?	?	?			?
Temp	•		•			○	○	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	?	?	?	?	•	•	?
Altitude	•	•											•	•	•	•	•	•	•	•	•	•	•	•	?	?	?				?
MM																															
Sanitation					•									•	•	○		•	•	•	•					•		•	•	•	○
Soil drench				•														•	•	•	•					?				•	•
MAT ME	○	○																		•		•		•	•	•	•				
BAT	○	○						•						○	○		?	•	•	•	?	•		•	•	•	•	•			
SIT	•	•					•																	•	•	•		•			
Cold T	○	•											•					•	•	•	•								•	•	
Heat T	○	•											•					•	•	•	•								•	•	
Irradiation													•					•	•	•									?	?	
Fumigation	•	•											•					•	•	•									?	?	
CF																															
Cultivar	•	•	•						•	•	•	•		•	•	•		•	•	•	?	?	?		?	?	?	?	?	?	
Fruit set	•	•	•	•			○						•			•		•	•	•		•	•					?			
Fruit Period	•	•	•	•			○						•			•		•	•	•		•	•					•			
Harvest t	•	•	•	○									•	•	•			•	•	•		?	?			?		?			
Wild hosts	•	•	•				?											•	•	?	?	•	•					•	?	?	
OFF& NE																															
Ceratitis	•	•	•	•	•		•		•	•	•	•	•	•	•	•	•				•					?		•			
Dacus	•	•	•	•	•		•		•	•	•	•	•	•	•	•	•				•					?		?			
ME.	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	?				•					?		?			
NE/P				•	•		?										?	•	•	•						•	?		?	?	?
B. invadens																															
Imm	•	•	•			•	•						?	•	•	•	•							•		•					
Emi	•	•	•										•	•	•	•	•							•		•					
Mating S						•	•	•														•	•			•	•		•		
Sex ratio	?	?	?			•		•														•	•			•					?
Adult de	?	?	?	•	?	•	•	•													•	•	•	•	•						
Adult d	?	?	?			•	•														•							•			
Oviposit	?	?		•			•	•					•	•	•	•	•	•	•	•	•						•		•		
Egg		•		•					•	•	•						?				•				•			•		•	
Larvae		•		•	•				•	•	•						?				•								•		•
Pupae	?	?	?	○	•																				?					•	

Figure 4.5: Interaction matrix that environmental factors, cropping systems and control may have on *Bactrocera invadens*. Climatic factors, management factors, crop factors, other fruit flies, natural enemies and parasitoids and biological factors of *B. invadens* were used to indicate possible interactions.

Matrix factors and key:
RH- Relative humidity, Temp- temperature, Altitude
MM- Management measures
MAT ME- male annihilation technique with methyl eugenol, BAT- bait application technique, SIT- sterile insect technique, Cold T- cold treatment, Heat T- heat treatment, irradiation, fumigation
CF- crop factors
OFF&NE- other fruit fly species and natural enemies and parasitoids
Ceratitis sp, *Dacus* sp, ME sp- other exotic methyl eugenol respondent fruit flies, NE/P- natural enemies and parasitoids
B. invadens- Biological factors for *B. invadens*
Imm- Immigration , Emi- Emigration , Mating S- mating success, Sex ratio, Adult de-adult density, Adult d-adult duration, Oviposition, Egg-egg survival, Larvae-larvae survival, Pupae-pupae survival.

It should be applied within an IPM system taking other control measures into consideration and may be effectively applied in combination with a suitable parasitoid (Vayssières *et al.,* 2009b). Environmental factors such as relative humidity and temperature and rainfall can play an important role in combination with control measures.

The post harvest measures indicated in the matrix may not be suitable for control in the production areas but may be suitable to be used before fruit is made available to the fresh produce market or as a treatment at processing plants of waste material or a treatment before processing takes place. Post harvest treatments will however have an influence on indigenous fruit fly eggs, larvae and parasitized eggs. Post harvest measures include cold and heat treatment, fumigation and irradiation. Cold treatment which would be effective against *C. capitata* would also be effective against *B. invadens* (Grout *et al.,* 2011; Hallman *et al.,* 2011) indicating that post harvest treatments designed for a specific fruit fly species would also interact with other insect species. Irradiation may be effective but is expensive and the treatment plants need to be able to adapt to effectively treat large volumes. This is a viable alternative to methyl bromide fumigation and is widely applicable with less damage to fruit quality (Hallman, 1999).

4.4.4.2. Research needs identified from the interaction matrix.

The matrix indicated several areas where more research may be needed.

4.4.4.2.1 Climate

All previous climatic modelling focused on climatic data collected from areas on the African continent where *B. invadens* was detected. No climatic modelling was done with detection data of *B. invadens* from areas in South Africa. The matrix identified that more research would be needed in terms of the effect climatic factors such as relative humidity, temperature and altitude would have on population dynamics in terms of sex ratio, adult duration, oviposition and the survival of pupae. This may provide critical information with regard to the possible climatic conditions *B. invadens* would need to establish and where in South Africa this pest could establish and if natural barriers potentially exist. It would also provide seasonal data which would allow predictions of population fluctuations, dispersal rate and range. Climatic modelling should be conducted with data from detection records in South Africa, other African countries as well as from areas where *B. invadens* occurs naturally.

The following interactions have been identified.

a. Relative humidity - sex ratio, adult density, adult duration, oviposition, pupae and mating success

b. Temperature – sex ratio, adult density, adult duration, oviposition, pupae and mating success

c. Altitude - sex ratio, adult density, adult duration, oviposition, pupae and mating success

4.4.4.2.2 Management

The impact that several management measures may have on different life stages of *B. invadens*, e.g. wild hosts, natural enemies and parasitoids have been identified. Soil drench has not been implemented by the NPPOZA during the eradication program followed in the PRA, as no agricultural chemical was registered to be utilised for it, but could potentially assist with the eradication especially where other methods such as protein bait sprays are less practical. It may be difficult to follow a bait application program in an urban area such as a town or a small village. Soil drenching may kill sufficient numbers of emerging adult fruit flies. The impact of a bait application program on natural enemies and parasitoids such as a cover spray over natural vegetation is not known. It potentially would be ideal to include natural vegetation in a BAT program but it could also have a negative effect on the natural enemies and parasitoid populations which may in the long run cause a need to use unnecessary high volumes of pesticides. Specific fumigation and irradiation schedules for *B. invadens* have not been determined yet on any hosts. Although generic schedules are commonly practiced, it would provide additional confidence to allow trade with non infested countries if specific schedules were available. The larval and/or egg survival rates should be determined for each cultivar presented to be tested to ensure the post harvest treatment applied is lowering the risk to the appropriate level of protection of that of the importing country.

The following relationships have been identified:

a. Soil drench- adult density

b. BAT- wild hosts, natural enemies and parasitoids

c. Irradiation- egg survival, larval survival

d. Fumigation- egg survival, larval survival

4.4.4.2.3 Crop factors

Uncertainties resulted from host status, host preference and specific susceptibility periods of many cultivars in relation to *B. invadens* which should have a high priority for further research. Control measures such as harvesting time, specific time frames for bait sprays can be optimised when host status is determined and may not be necessary for certain hosts (Aluja & Mangan, 2008; De Graaf, 2010). The susceptibility of different cultivars of a host to *B. invadens* provides information which is important to ensure good management practices. Fruit fly population numbers differ seasonally and as cultivars may have different ripening times and fruiting periods, it may be less or more susceptible to infestation depending on the population numbers of *B. invadens*. The harvesting time and period of available fruit may also be influenced by the cultivar planted. Fruit of some cultivars may not yet be susceptible to oviposition by the time it was harvested. This would reduce the risk of infested fruit being harvested which would ensure more trading possibilities with foreign markets. It will also reduce the risk that the pest would accidentally be spread to a new unaffected area. Successful oviposition may occur on some cultivars but due to the ripening stage of the fruit and the defence mechanisms of the cultivar, egg and larval survival may be influenced. Egg and larval survival may have an influence on parasitoid abundance. Parasitoids and natural enemies may not be abundant in orchards of some cultivars due to other pest control programs or the cultivar itself. *B. invadens* has been introduced twice into the study area and subsequently eradicated. Such research was therefore not possible in South Africa. The availability of susceptible cultivars may also influence the rate that *B. invadens* immigrate into an area or the rate it emigrates from an area. Fruit flies will generally stay in an area as long as their suitable hosts are available. The availability of suitable cultivars may also influence sex ratio and adult density in a specific area. The availability of wild hosts in the production area may influence the success of control mechanisms such as the bait application technique. It may also serve as a small area where population numbers can increase due to higher egg and larval survival rates. Exotic fruit flies may be more likely to enter and multiply in such areas undetected which would increase their survival potential.

Proper host plant determination for *B. invadens* is therefore critical in terms of susceptibility for oviposition on fruit of plant species, cultivars and ripening stage. This should be a research priority for South Africa but may only be implementable in countries with established colonies. The impact that this data would have on management measures implemented, period of fruit available, and harvesting time could be vital to ensure more trade alternatives.

The following relationships have been identified:

a. Cultivar- natural enemies and parasitoids, immigration, emigration, sex ratio, adult density, oviposition, egg survival, larval survival

b. Fruit set time- oviposition

c. Harvest time- immigration, emigration, adult density, and oviposition

d. Wild hosts- BAT, ME exotics, natural enemies and parasitoids, egg survival, larval survival

4.4.4.2.4 Indigenous fruit flies and natural enemies

Bactrocera invadens is highly competitive and invasive in natural habitats. Species from the genera *Ceratitis* and *Dacus* were found to be competing for the same commercial and some wild hosts as *B. invadens*. With the establishment of *B. invadens* in an area the population numbers of the local fruit flies may be reduced and local fruit fly populations may be displaced in some areas. The presence of *B. invadens* in an area may also influence the establishment of other exotic fruit flies attracted to the same lures. The presence of natural enemies and parasitoids in an area may also influence the adult density, egg, larval and pupal survival of *B. invadens*.

The establishment or regular reintroductions of *B. invadens* into different areas in South Africa are inevitable. It is therefore important that preferable indigenous natural enemies and parasitoids are identified. It is also important to allow the augmentative release of parasitoids as well as classical biological control release of either indigenous or exotics in the origin for alternative control mechanisms (Waage & Hassell, 1982). This would require intensive research in countries where *B. invadens* has established in terms of parasitoids applied, as well as in South Africa with regard to locally available natural enemies and parasitoids.

The following relationships have been identified:

a. *Ceratitis* spp. - Adult density of *B. invadens*

b. *Dacus* spp.- Adult density of *B. invadens*

c. ME exotics - Adult density of *B. invadens*

d. Natural enemies and parasitoids- Adult duration, egg survival, larval survival, pupae survival, adult density.

4.4.5 Conclusions

Corrective actions to eradicate this pest should be implemented as the probability is high that more and more frequent introductions of *B. invadens* may continue in the study area. These should be included as part of a corrective action plan by the NPPOZA.

Eradication measures, although mandatory, can be combined with cultural measures such as orchard or field sanitation as part of an IPM system to implement corrective actions. IPM systems should include the possibility of making use of natural enemies and parasitoids.

Importation and local production of identified biological control agents should be initiated since these agents could disperse across borders into already infested areas.

The application of area wide control measures should be as environmentally friendly as possible. This can be ensured by the placement of male annihilation blocks and bait sprays with GF120 instead of an organophosphate/ protein mixture.

The continuous interaction with local communities is essential to ensure successful application of corrective actions as well as quarantine measures. Awareness programs should be increased to local community level which should include information pamphlets in local languages and community radio broadcasts.

Research is needed to establish the host status of *B. invadens* regarding commercial hosts in the area to optimise harvesting times as well as the application of other cultural control methods such as fruit bagging. The probability of establishment of *B. invadens* in different climatic conditions in South Africa should be researched. Climatic modelling with available host records of detections in South Africa should be done in conjunction with records elsewhere on the continent and in Asia. The impact *B. invadens* may have on the natural fauna may also be important and should be followed up.

CHAPTER 5: FINAL CONCLUSIONS.

The findings of the pest risk assessment in Chapter 3 are based on analysis of relevant available scientific and other appropriate literature. By comparing the different factors that may contribute to the probability to enter, spread and establish in the Musina area to each other and to the result of the detection survey an overall risk probability could be determined. The probable economic and environmental consequences that this pest could pose were further evaluated and the overall risk determined. The overall risk of the species initiated qualitative risk assessment of *B. invadens* was determined to be high to enter and establish in the Musina area and the rest of South Africa with a probability of severe economic consequences.

It was determined that *B. invadens* is a high risk invasive species with the potential to cause serious direct and indirect economic damage to nearly all fruit growing regions in South Africa. It also may have an environmental impact as it may displace indigenous fruit flies from an area. This pest is capable of dispersing long distances, both naturally and through the artificial movement of infested host materials, such as fruit.

B. invadens was detected in 2010 as well as 2011 during the three years of surveillance from 2009 to 2011 with 42 weeks of zero detection between the last detection in 2010 and the first detection in 2011. This indicates that the pest was introduced into the area on two separate occasions.

The risk management measures proposed in Chapter 4 should achieve appropriate protection against the importation of fresh fruit and vegetable hosts of *B. invadens*. Various risk management measures may be suitable to manage the risk of *B. invadens* in the pathways associated with the import of host material into South Africa.

Eradication of *B. invadens* may be feasible in cases of low population numbers such as the founder populations which were detected in 2010 and 2011 which made eradication attempts successful as indicated in Chapter 3.

However, the probability is high that more incursions of *B. invadens* will occur as demonstrated with the detection survey. Therefore import regulations should aim to manage the risk of the pest in the exporting country to avoid the presence of *B. invadens* in imported host material. The risk that *B. invadens* will enter with infested fruit carried by passengers in cabins of vehicles is high and border post control systems and legislation should be amended to manage the risk.

Management measures in affected areas in South Africa are necessary to slow population growth on farm level and to eradicate existing populations. Effective management of *B. invadens* involves an integrated approach which includes the application of corrective and quarantine actions. *B. invadens* should be managed on an area wide control basis which can be implemented in a systems approach. This will require a high level of industry and community engagement in a joint approach between DAFF, fruit industry associations, provincial government, local government and local communities. This will also require amendments to the existing legislation and action plans.

A systems approach which is integrated with existing IPM systems to control *B. invadens* could provide quarantine security to expand exports of fruit to new markets as long as continuous surveillance is maintained to serve as an early warning system to new incursions or to monitor population levels.

The pest risk assessment identified several research needs of which the most important may be the need to conduct proper host status research for each documented host of *B. invadens*.

CHAPTER 6: REFERENCES

ABANDA, F. N., QUILICI, S., VAYSSIÈRES, J. F., KOUODIEKONG, L., & WOIN, N. 2008. Inventory of fruit fly species on guava in the area of Yaounde, Cameroon. *Fruits* 63: 19-26.

ABOAGYE. P. 2009. *Impact of the current postharvest storage practices on the quality of Ghana mango for export*. Thesis (Masters Degree in Agricultural Production). Chain management, specialization. Post harvest technology and logistics. Larenstein University of Applied Sciences. Wageningen. The Netherlands.

ACOCKS, J.P.H. 1988. Veld types of South Africa, 3rd Edition. *Memoirs of the Botanical Survey of South Africa* No. 57. Pretoria: Government Printer, 146 pp.

ACTS *see* ANONYMOUS.

ALUJA, M. & MANGAN, R.L. 2008. Fruit fly (Diptera: Tephritidae) host status determination: Critical conceptual and methodological considerations. *Annual Review of Entomology* 53: 449-472.

ANNECKE, D.P. & MORAN, V.C. 1982. *Insects and mites of cultivated plants in South Africa*. Butterworths, Durban. pp 308.

ANONYMOUS. 1983. Agricultural Pest Act, 36 of 1983.

ANONYMOUS. 2009. Agricultural Pests Act (36/1983): Control Measures R110: Amendment. (Proclamation No. R.1148, 2009) *Government Gazette*, 32781, 12 Dec.2009.

ARMSTRONG, J.W. 1991. Sharwill Avocado: Quarantine security against fruit fly (Diptera: Tephritidae) infestation in Hawaii. *Journal of Economic Entomology* 84:1398-1315.

BAKER, R. & MACLEOD, A. 2005. Pest Risk Assessments: Tools, Resources and Key Challenges, *In* Hedley, B., *ed***.** Secretariat of the International Plant Protection Convention. Identification of risks and management of invasive species using the IPPC framework. Proceedings of the workshop on invasive species and the International Plant Protection Convention, Braunschweig, Germany, 22-26 September 2003. 106-109. Rome, Italy FAO.

BARNES, B.N. & VENTER, J.-H. 2008. The South African fruit fly action plan- area-wide suppression and exotic species surveillance. *In* Sugayama, R. L., Zucchi, R. A., Ovruski, S.

M. & Sivinski, J*., eds.* Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2006. 271-283. Biofabrica Moscamed Brasil. Salvador, Bahia, Brazil.

BARTLETT, P.W. 1996. Tephritid fruit flies- a quandary for international plant quarantine. *In* Brighton Crop Protection Conference Pest and Diseases Vol 3, 18 –21 November 1996. 1153-1160. Brighton United Kingdom.

BATEMAN, M.A., DREW, R.A.I. & HOOPER, G.H.S. 1978. *Economic Fruit Fly of the South Pacific Region*. Brisbane, Queensland Department of Primary Industries, 130 p.

CANTRELL, B., CHADWICK, B. & CAHILL, A. 2002. *Fruit fly fighters: eradication of the papaya fruit fly.* Series: SCARM Report No. 81. Commonwealth of Australia and each of its States and Territories. CSIRO Publishing, Collingwood, Australia.

CGA. 2010. Annual Report 2010. Citrus Growers' Association of Southern Africa. http://www.cga.co.za/site/files/5438/CGA%20ANNUAL%20REPORT%202010%20web.pdf. Date of access: 12 June 2011.

CHEGEH, B.K., WATURU, C.N., WEPUKHULU, S.B. & MBAKA J.N. 2010. Prolonging avocado shelf life using ethylene synthesis inhibitor, 1-methylcyclopropene. *In* 10th KARI Biennial Scientific Conference 6 May 2010. Kenya Agricultural Research Institute

CLARKE, A.R., ARMSTRONG, K.F., CARMICHAEL, A.E., MILNE, J.R., RAGHU, S., RODERICK, G.K. & YEATES, D.K. 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology* 50: 293-319.

CLIFT, A. & MEATS, A. 2002. When does zero catch in a male lure trap mean no Tephritid flies in the area? *In*: Barnes, B., *ed.* Proceedings of the 6th International fruit fly symposium*. 6-10* May 2002.183-188. B Istec Scientific Publications Irene. South Africa.

CLIFT, A. & MEATS, A. 2005. Use of a Bayesian Belief Network to identify situations that favour Fruit Fly incursions in inland SE Australia. *In:* Zerger, A. and Argent, R.M. *eds* Modsim 2005. International congress on modelling and simulation; modelling and simulation society of Australia and New Zealand. 170-176. http://www.mssanz.org.au/modsim05/papers/clift.pdf. Date of access: 30 March 2009.

COPELAND, R.S., WHARTON, R.A., LUKE, Q., DE MEYER, M., LUX, S., ZENZ, N., MACHERA, P. & OKUMU, M. 2006. Geographic distribution, host fruit, and parasitoids of African fruit fly pests *Ceratitis anonae, Ceratitis cosyra, Ceratitis fasciventris*, and *Ceratitis rosa* (Diptera: Tephritidae) in Kenya. *Annals of the Entomological Society of America* 99: 261-278.

CORREIA, A.R.I., REGO, J.M. & OLMI, M. 2008. A pest of significant economic importance detected for the first time in Mozambique: *Bactrocera invadens* Drew, Tsuruta & White (Diptera: Tephritidae: Dacinae). *Bollettino di Zoologia Agraria e di Bachicoltura* 40: 9-13.

DAFF. 2010a. Economic Review of the South African Agriculture. Directorate Statistics and Economic analysis. Department of Agriculture, Forestry and Fisheries. http://www.daff.GOV.za/docs/statsinfo/Eco2010.pdf. Date of access: 12 June 2011.

DAFF. 2010b. *A profile of the South African citrus market value chain*. Directorate Marketing. Department of Agriculture, Forestry and Fisheries. http://www.daff.gov.za/docs/AMCP/CitrusMVCP2010-11.pdf. Date of access: 12 June 2011.

DAFF. 2010c. *A profile of the South African mango market value chain.* Directorate Marketing. Department of Agriculture, Forestry and Fisheries. http://www.daff.gov.za/docs/AMCP/MangoMVCP2010-11.pdf. Date of access: 12 June 2011.

DAFF. 2010d. *A profile of the South African tomato market value chain.* Directorate Marketing. Department of Agriculture, Forestry and Fisheries. http://www.daff.gov.za/docs/AMCP/TomatoMVCP2010-11.pdf
Date of Access. 12 December 2011.

DE GRAAF, J. 2010. Host Status of Avocado ('Hass') to *Ceratitis capitata*, *Ceratitis rosa,* and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa. *Journal of Economic Entomology* 102: 1448-1459.

DE HOOP, B. 2005. Pest Risk Analysis: Global Harmonization Benefits Biodiversity, pp94-97, *In* Hedley. B., *ed.* IPPC Secretariat.2005. Identification of risks and management of invasive species using the IPPC framework. Proceedings of the workshop on invasive species and the International Plant Protection Convention, Braunschweig, Germany, 22-26 September 2003. Rome, Italy FAO.

DE MEYER, M., MOHAMED, S. & WHITE, I.M. 2012. *Invasive fruit fly pests in Africa*. http://www.africamuseum.be/fruitfly/AfroAsia.htm. Date of access: 18 October 2012.

DE MEYER, M., ROBERTSON, M.P., MANSELL, M.W., EKESI, S., TSURUTA, K., MWAIKO, W., VAYSSIERES, J. & PETERSON, T. 2010. Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae). *Bulletin of Entomological Research* 100: 35-48.

DoA. 2006. *Protocol of phytosanitary equirements for the export of citrus fruit from South Africa to China between the Department of Agriculture of the Republic of South Africa and the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China*. Departement of Agriculture. http://www.nda.agric.za/doaDev/sideMenu/plantHealth/docs/Protocol_citrusfruit_USA.pdf. Date of access: 5 June 2011.

DoA. 2007. *Protocol of phytosanitary requirements for the export of apple fruit from China to South Africa between the Department of Agriculture of the Republic of South Africa and the general Administration of Quality Supervision, Inspection and Quarantine of The People's Republic of China.* Department of Agriculture Forestry and Fisheries: http://www.nda.agric.za/doaDev/sideMenu/plantHealth/docs/Protocol_%20import_apple_China-SA.pdf. Date of access: 12 June 2011.

DoA. 2008. *Work Plan for the USDA preclearance inspection and cold treatment of South African citrus fruit designated for export to the United States of America*. Department of Agriculture. http://www.nda.agric.za/doaDev/sideMenu/plantHealth/docs/Protocol_citrusfruit_USA.pdf. Date of access: 5 June 2011.

DREW, R.A.I., TSURUTA, K. & WHITE, I.M. 2005. A new species of pest fruit fly (Diptera: Tephritidae) from Sri Lanka and Africa. *African Entomology* 13:149-154.

DUMBA, S. & CHIRISA, I. 2010. The plight of illegal migrants in South Africa: a case study of Zimbabweans in Soshanguve extension 4 And 5. *International Journal of Politics and Good Governance* 1:1-20.

DUYCK, P.F., STERLIN, J.F. & QUILICI, S. 2004a. Survival and development of different life stages of *Bactrocera zonata* (Diptera: Tephritidae) reared at five constant temperatures compared to other fruit fly species. *Bulletin of Entomological Research* 94: 89–93.

DUYCK, P.F., DAVID, P, & QUILICI, S. 2004b. A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae).Ecological Entomology, 29: 511-520.

EKESI, S. & BILLAH, M.K. 2006. *A Field Guide to the management of economically important Tephritid fruit flies in Africa*. ICIPE Science Press Nairobi, Kenya. 206 pp.

EKESI, S., BILLAH, M.K., PETERSON, W.N., LUX, S.A. & RWOMUSHANA, I. 2009. Evidence for competitive displacement of *Ceratitis cosyra* by the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) on mango and mechanisms contributing to the displacement. *Journal of Economic Entomology* 102: 981-991.

EKESI, S., NDERITU, P. W. & RWOMUSHANA, I. 2006. Field infestation, life history and demographic parameters of the fruit fly *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Bulletin of Entomological Research* 96: 379-386.

EPPO. 2010. *Report of a Pest Risk Analysis for Bactrocera invadens. EPPO Standards10-16120.* European and Mediterranean Plant Protection Organization Organisation Europeenne Et Mediterraneenne Pour La Protection Des Plantes *Paris,France .* http://www.eppo.org/QUARANTINE/Pest_Risk_Analysis/PRA_documents.htm. Date of Access: 2 March 2011.

FAKAYODE, S.B., OMOTESHO, O.A., BABATUNDE, R.O., & MOMOH, A.A. 2010. The sweet orange market in Nigeria, How Viable?. *Journal of Agricultural Biology*. 6: 395-400.

FAO. 2009. International Standards for Phytosanitary Measures 1-32. *Secretariat of the International Plant Protection Convention*, Rome FAO, Italy. https://www.ippc.int/servlet/BinaryDownloaderServlet/124047_2009_ISPMs_book_Engl.doc?filename=1187683730555_ISPMs_1to29_2009_En_with_convention.doc&refID=124047. Date of Access: 1 July 2011.

FAO. 2011. International Standards for Phytosanitary Measures No. 26 (2006) Appendix 1. *Secretariat of the International Plant Protection Convention*, Rome FAO, Italy. https://www.ippc.int/servlet/BinaryDownloaderServlet/124047_2009_ISPMs_book_Engl.doc?filename=1187683730555_ISPMs_1to29_2009_En_with_convention.doc&refID=124047. Date of Access: 1 July 2011.

FAO/IAEA. 2000. *Action Plan: Peach Fruit Fly, Bactrocera zonata* (Saunders). Joint FAO/IAEA Division, Vienna, Austria. FAO/IAEA Division, Vienna, Austria. http://www.iaea.org/programmes/nafa/d4/ public/zonata-actionplan.pdf .Date of access: 30 March 2009.

FAO/IAEA. 2011. *Guidelines for implementing systems approaches for pest risk management of fruit flies.* Report and recommendations of the consultants group meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, June 7-11 2010. IAEA Vienna, Austria. http://www-naweb.iaea.org/nafa/ipc/public/IPC-systems-approach-2011.pdf. Date of Access: 25 August 2011.

FLOREC, V., SADLER, R. & WHITE, B. 2010. *Area-Wide Management of Fruit-Flies: What are the Costs and the Benefits?*, Working Paper 1008 Australia, Crawley, Australia.

FOLLETT, P.A. & VARGAS, R.I. 2010. A systems approach to mitigate oriental fruit fly risk in 'Sharwill' avocados exported from Hawaii. *Acta Horticulturae* 880: 439-445.

FOLLETT, P.A. & NEVEN, L.G. 2006. Current trends in quarantine entomology. *Annual Review of Entomology* 51: 359–385.

FRAMPTON, E.R. 2000. An overview of quarantine for fruit flies, *In*: Tan, K. H *ed.* Proceedings: Area-wide control of fruit flies and other insect pests. Joint proceedings of the international conference on area-wide control of insect pests, May 28-June 2, 1998 and the fifth international symposium on fruit flies of economic importance, June 1-5, 1998. pp 381-388. Penerbit Universiti Sains Malaysia, Penang, Malaysia.

GOERGEN, G., VAYSSIÈRES, J.F., GNANVOSSOU, D., & TINDO, M. 2011. *Bactrocera invadens* (Diptera: Tephritidae), A new invasive fruit fly pest for the Afrotropical region: Host plant range and distribution in West and Central Africa. *Environmental entomology* 40: 844-854.

GRIFFIN, R.L. 2000. Trade issues and area-wide pest management. *In* K.H. Tan. *ed*. Proceedings: Area-wide control of fruit flies and other insect pests. International conference on area-wide control of insect pests, and the 5th international symposium on fruit flies of economic importance, 28 May-5June 1998, pp 29-53, Penang, Malaysia. Penerbit University Sains Malaysia, Pulau Pinang, Malaysia.

GROUT, T.G., DANEEL, J.H., MOHAMED, S.A., EKESI, S., NDERITU, P.W., STEPHEN, P.R. & HATTINGH, V. 2011. Cold susceptibility and disinfestation of *Bactrocera invadens* (Diptera: Tephritidae) in oranges. *Journal of Economic Entomology* 104:1180-1188.

HALLMAN, G.J. 1999. Ionizing radiation quarantine treatments against tephritid fruit flies. *Postharvest Biology and Technology* 16: 93–106.

HALLMAN, G.J., MYERS, S.W., JESSUP, A.J. & ISLAM, A. 2011. Comparison of in vitro heat and cold tolerances of the new invasive *Bactrocera invadens* (Diptera: Tephritidae) with three known tephritids. *Journal of Economic Entomology* 104: 21-25.

HANCOCK, D.L., OSBORNE, R., BROUGHTON, S., & GLEESON, P. 1998. Eradication of *Bactrocera papayae* (Diptera: Tephritidae) by male annihilation and protein baiting in Queensland, Australia. *In* K.H. Tan *ed*. Proceedings: Area-wide control of fruit flies and other insect pests. International conference on area-wide control of insect pests, and the 5th International symposium on fruit flies of economic importance, 28 May to 5 June 1998. Penang, Malaysia. Penerbit University Sains Malaysia, Pulau Pinang, Malaysia. pp. 381–388.

HANNA, R. 2005. The Asian fruit fly *Bactrocera invadens* in West and Central Africa: distribution, host range and seasonal dynamics. http://esa.confex.com/esa/2005/techprogram/paper_23053.htm. Date of access: 12 December 2011.

HEE, A.K.-W & Tan, K.-H. 2004. Male sex pheromonal components derived from Methyl Eugenol in the hemolymph of the fruit fly *Bactrocera papayae. Journal of Chemical Ecology* 30: 2127-2138.
http: //www.eppo.org/QUARANTINE/Pest_Risk_Analysis/PRA_intro.htm. Date of Access: 23 January 2011

HICHAAMBWA, M., & TSCHIRLEY, D.L. 2006. *Zambia horticultural rapid appraisal: understanding the domestic value chains of fresh fruits and vegetables (No. 54476)*. Michigan State University, Department of Agricultural, Food, and Resource Economics.

HUXHAM, K.A. 2002. A Unique fruit fly monitoring and control system-Australia's frontline defence,. *In*: Barnes., *ed*. Proceedings of the 6th International Fruit fly Symposium. 331-334 B Istec Scientific Publications Irene.

IAEA. 2000. Action Plan: Peach Fruit Fly, *Bactrocera zonata* (Saunders). Joint FAO/IAEA Division, Vienna, Austria. http://www.iaea.org/programmes/nafa/d4/ public/zonata-actionplan.pdf. Date of access: 30 March 2009.

IAEA, 2003. Trapping Guidelines for Area-Wide Fruit Fly Programmes, Joint FAO/IAEA Division, Vienna, Austria. http://www-naweb.iaea.org/nafa/ipc/public/ trapping-web.pdf. Date of access: 30 March 2009

IPPC. 2010. First detection of *Bactrocera invadens* in Botswana. IPPC Official Pest Report, No. BWA-01/2. Rome, Italy: FAO. https://www.ippc.int/IPP/En/default Date of access: 14June 2011.

JANG, E.B., & H.R. MOFFIT. 1994. Systems approaches to achieving quarantine security, pp. 224-237. *In* J. L. Sharp and H. J. Hallman *eds*. *Quarantine treatments for pests of food plants*. Westview Press, Boulder, CO.

JANG, E.B., MAU, R.F., VARGAS, R.I. & MCINNIS, D.O. 2006. Exporting fruit from low fruit fly prevalence zones with a multiple mitigation systems approach. *Food and Fertilizer Technology Centre Extension Bulletin* 584.

KHAMIS, F.M., KARAM, N., EKESI, S., DE MEYER, M., BONOMI, A., GOMULSKI, L.M., SCOLARI, F., GABRIELI P., SICILIANO P., MASIGA D., KENYA E.U., GASPERI G., MALACRIDA A. R., & GUGLIELMINO, C. R. 2009. Uncovering the tracks of a recent and rapid invasion: the case of the fruit fly pest *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Molecular Ecology*, 18: 4798-4810.

KLUNGNESS, L.M., JANG, E.B., MAU, R.F.L., VARGAS, R.I., SUGANO, J.S. & FUJITANI, E. 2005. New sanitation techniques for controlling Tephritid Fruit Flies (Diptera: Tephritidae) in Hawaii. *Journal of Applied Sciences and Environmental Management* 9: 5-15.

KWASI, W. 2008. Assessment of Fruit Fly Damage and Implications for the dissemination of management practices for mango production in the upper West region of Ghana. *Journal of Developments in Sustainable Agriculture* 3: 117-134.

LIEBHOLD, A. M. & TOBIN, P. C. 2008. Population ecology of insect invasions and their management. *Annual Review of Entomology* 53: 387-408.

LIU, L., LIU, J., WANG, Q., NDAYIRAGIJE, P., NTAHIMPERA, A., NKUBAYE, E., YANG, Q & Li, Z. 2011. Identification of *Bactrocera invadens* (Diptera: Tephritidae) from Burundi, based on morphological characteristics and DNA barcode. *African Journal of Biotechnology* 10: 13623-13630.

LOPIAN, R. 2003. The International Plant Protection Convention and invasive alien species. Pp 6-16, *In*: Hedley, B.*ed* . Identification of risks and management of invasive species using the IPPC framework. Proceedings of the workshop on invasive species and the International Plant Protection Convention, Braunschweig, Germany, 22-26 September 2003. IPPC Secretariat FAO, Rome, Italy.

LUX, S.A., COPELAND, R., WHITE, I.M., MANRAKHAN, A. & BILLAH, M. 2003. A new invasive fruit fly species from the *Bactrocera* dorsalis group detected in East Africa. *Insect Science and its Application* 23:355-361.

MANGAN, R.L., FRAMPTON, E.R., THOMAS, D.B. & MORENO, D.S. 1997. Application of the maximum pest limit concept to quarantine security standards for the Mexican fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology* 90:1433-1440.

MANRAKHAN, A., VENTER, J.H. & HATTINGH, V. 2009. *Bactrocera invadens* Drew Tsuruta and White: The African Invader fly Action Plan. http://www.nda.agric.za/doaDev/sideMenu/plantHealth/docs/SABIFFactionPlan.pdf. Date of access: 20 September 2011.

MANRAKHAN, A., HATTINGH, V., VENTER, J-H. & HOLTZHAUSEN, M. 2011. Eradication of *Bactrocera invadens* (Diptera: Tephritidae) in Limpopo Province, South Africa. *African Entomology* 19: 650–659.

MEATS, A. 1998. The power of trapping grids for detecting and estimating the size of invading propagules of the Queensland fruit fly and risk of subsequent infestation. *General and Applied Entomology* 28: 47-55.

MOHAMED, S. A., EKESI, S. & HANNA, R. 2010. Old and new host-parasitoid associations: parasitism of the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) and five African fruit fly species by *Fopius arisanus*, an Asian opiine parasitoid, *Biocontrol Science and Technology* 20: 183-196.

MUMFORD, J.D. 2005. Application of benefit/cost analysis to insect pest control using the sterile insect technique. pp481-489, *In*: V.A. Dyck, J. Hendrichs & A.S. Roberson *eds.*, *Sterile insect technique. Principle and practice in area-wide integrated pest management*. Springer. The Netherlands.

MURAJI, M & NAKAHARA, S. 2002, Discrimination among pest species of *Bactrocera* (Diptera: Tephritidae) based on PCR-RFLP of the mitochondrial DNA. *Applied Entomology and Zoology* 37:437–446

MWATAWALA, M. W., DE MEYER, M., MAKUNDI, R. H. & MAERERE, A. P. 2006a. Biodiversity of fruit flies (Diptera: Tephritidae) in orchards in different agro-ecological zones of the Morogoro region, Tanzania. *Fruits* 61: 321-332.

MWATAWALA, M. W., DE MEYER, M., MAKUNDI, R. H. & MAERERE, A. P. 2006b. Seasonality and host utilization of the invasive fruit fly, *Bactrocera invadens* (Dipt., Tephritidae) in central Tanzania. *Journal of Applied Entomology* 130: 530-537.

MWATAWALA, M. W., DE MEYER, M., MAKUNDI, R. H., & MAERERE, A. P. 2009. Host range and distribution of fruit-infesting pestiferous fruit flies (Diptera, Tephritidae) in selected areas of Central Tanzania*. Bulletin of Entomological Research* 10: 1-13.

MWATAWALA, M. W., WHITE, I. M., MAERERE, A. P., SENKONDO, F. J. & DE MEYER, M. 2004. A new invasive *Bactrocera* species (Diptera: Tephritidae) in Tanzania. *African Entomology* 12: 154-156.

NDIAYE, M., DIENG, E. O. & DELHOVE, G. 2007. Population dynamics and on farm fruit fly integrated pest management in mango orchards in the natural area of Niayes in Senegal. *Pest Management in Horticultural Ecosystems* 14:1-8.

NORTON, G.A. & MUMFORD, J.D. 1994. Descriptive techniques. pp 23-42, *In*: Norton, G. A. & Mumford, J.D. *eds*, *Decision tools for pest management*. CAB International, Wallingford, UK.

OGAUGWU, C., WILSON, D., COBBLAH, M., & ANNOH, C. 2012. Gamma radiation sterilization of *Bactrocera invadens* (Diptera: Tephritidae) from southern Ghana. *African Journal of Biotechnology*, 11: 11315-11320.

PEBERDY, S. 2000. Mobile entrepreneurship: Informal sector cross-border trade and street trade in South Africa. *Development Southern Africa* 17: 201-219.

PEÑA, J.E., MOHYUDDIN, A.I. & WYSOKI, M. 1998. A review of the pest management situation in mango agroecosystems. *Phytoparasitica* 26:129-148.

PHELONG, P. 2005. Contingency planning for plant pest incursions in Australia, *In* Hedley, B., *ed*. Secretariat of the International Plant Protection Convention. Identification of risks and management of invasive species using the IPPC framework. Proceedings of the workshop on invasive species and the International Plant Protection Convention, Braunschweig, Germany, 22-26 September 2003. pp.166-174. Rome, Italy FAO.

PINERO, J.C., MAU, R.F.L. & VARGAS, R.I. 2009. Managing oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae), through spinosad-based protein bait sprays and sanitation in papaya orchards in Hawaii. *Journal of Economic Entomology* 102:1123–1132.

QUINLAN, M.M. 2002. Trends in phytosanitary standards: potential consequences on fruit fly control. *In* Barnes, B., *ed.* Proceedings of the 6th International Fruit fly Symposium. pp195-200. B Istec Scientific Publications Irene. South Africa.

QUINLAN, M.M. & IKIN, R. 2009. PRATIQUE. An EU framework 7 research project: enhancements of pest risk analysis techniques. Deliverable No. 4.2: *A review of the application of systems approach to risk management in plant health.* 69pp. Available at: https://secure.fera.defra.gov.uk/pratique/index.cfm. Date of access: 26 August 2011.

RAMASODI, R.M. 2008. *Pest Risk Analysis on hand luggage at OR Tambo International Airport: A case study of flights from Cameroon, India and Kenya*. Thesis (M.Inst.Agrar.)(Plant Production (Agronomy) University of Pretoria, 2008. http://upetd.up.ac.za/thesis/available/etd-02112009-104221/unrestricted/dissertation.pdf. Date of access: 10 June 2011.

RUTHERFORD, M. C. & WESTFALL, R. H. 1994. *Biomes of Southern Africa - an objective categorization*. Memoirs of the Botanical Survey of South Africa 63. Botanical Research Institute, Department of Agriculture and Water Supply. Pretoria 54:1-98.

RWOMUSHANA, I., EKESI, S., GORDON, I. & OGOL, C. K. P. O. 2008a. Host plants and host plant preference studies for *Bactrocera invadens* (Diptera: Tephritidae) in Kenya, a new invasive fruit fly species in Africa. *Annals of the Entomological Society of America* 101:331-340.

RWOMUSHANA, I., EKESI, S., OGOL, C.K.P.O., & GORDON, I. 2008b. Effect of temperature on development and survival of immature stages of *Bactrocera invadens* (Diptera: Tephritidae). *Journal of Applied Entomology*, 132: 832-839.

SA Fruit farms http://www.safruitfarms.com/table-grapes.aspx Date of access: 20 September 2011.

SA Weather Service. 2011. *Weather data of the Musina area. Climatological information of the normal values, according to World Meteorological Organization (WMO) prescripts, based on monthly averages for the 30-year period 1961 – 1990*. http://old.weathersa.co.za/Climat/Climstats/MusinaStats.jsp. Date of access: 19 July 2011.

SOLOMON, H. 2010. Contemplating the impact of illegal immigrants on the Republic of South Africa (Working Paper), University of Pretoria*. International Journal of Politics and Good Governance* 1: 1-20.

STATISTICS SOUTH AFRICA. 2002. *Census of Agriculture Provincial Statistics 2002-Limpopo Financial and production statistics Report No. 11-02-10.* http://harvestchoice.org/sites/default/files/downloads/publications/RSA_2002_10_LP.pdf. Date of access: 19 July 2011.

STONEHOUSE, J., MUMFORD, J., POSWAL, A., MAHMOOD, R., MAKHDUM, H.A, CHAUDHARY, Z.M., BALOCH, K.N., MUSTAFA, G. & MCALLISTER, M. 2004. The accuracy and bias of visual assessments of fruit infestation by fruit flies (Diptera: Tephritidae). *Crop Protection* 23: 293–296.

STONEHOUSE. J.M., MUMFORD. J.D. & MUSTAFA, G. 1998. Economic losses to fruit flies(Diptera) in Pakistan. *Crop Protection* 11: 159-164.

TAN, K.-H. & NISHIDA, R. 2000. Mutual reproductive benefits between a wild orchid, *Bulbophyllum patens*, and *Bactrocera* fruit flies via a Floral Synomone. *Journal of Chemical Ecology* 26: 33-546.

TAN, K.-H., NISHIDA, R., & TOONG, Y.-C. 2002. Floral synomone of a wild orchid, *Bulbophyllum cheiri*, lures *Bactrocera* fruit flies for pollination. *Journal of Chemical Ecology* 28:1161-1172.

TAN, K.-H., TAN, L.T. & NISHIDA, R. 2006. Floral phenylpropanoid cocktail and architecture of *Bulbophyllum vinaceum* orchid in attracting fruit flies for pollination. *Journal of Chemical Ecology* 32: 112429-2441.

TAN, K. H., TOKUSHIMA, I., ONO, H. & NISHIDA, R. 2010. Comparison of phenylpropanoid volatiles in male rectal pheromone gland after methyl eugenol consumption, and molecular phylogenetic relationship of four global pest fruit fly species: *Bactrocera invadens*, *B. dorsalis, B. correcta* and *B. zonata*. *Journal of Chemical Ecology* 21: 25-33.

TAN. K.-H. 2006. Fruit fly pests as pollinators of wild orchids. *In*: Sugayama, R. L., Zucchi, R. A., Ovruski, S. M. & Sivinski, J. *eds.* Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2006. p195-206. Biofabrica Moscamed Brasil. Salvador, Bahia, Brazil.

THOMAS, D.B., HOLLER, T.C., HEATH, R.T. R., SALINAS, E.J. & MOSES, A. 2001. Trap-Lure Combinations for Surveillance of Anastrepha Fruit Flies (Diptera: Tephritidae). *Florida Entomologist* 84: 344.

TRADEMAP. 2011. *List of importing markets for a product exported by South Africa. Product : 0805 Citrus fruit, fresh or dried*. http://www.trademap.org/Country_SelProduct.aspx. Date of access: 19 July 2011.

USDA-APHIS. 2006. *Exotic fruit fly strategic plan. fy 2006-2010,* Fruit Fly Exclusion and Detection Programs 4700 River Road, Unit 137, United States Department of Agriculture, http://www.aphis.usda.gov/plant_health/plant_pest_info/fruit_flies/downloads/strategicplan06-19-06.pdf. Date of access: 13 April 2009.

USDA-APHIS. 2008. *Federal import quarantine order for host materials of Bactrocera invadens (Diptera: Tephritidae), invasive fruit fly species. 5.* http://www.aphis.usda.gov/import_export/plants/plant_imports/federal_order/downloads/*BactroceraInvadens*May2009.pdf. Date of access: 12 August 2011.

USDA-APHIS. 2011. *Treatment manual*. United States Department of Agriculture, Animal and Plant Health Inspection Service Plant Protection and Quarantine http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf. Date of access: 19 July 2011.

VAN MELE, P. VAYSSIÈRES, J-P. VAN TELLINGEN, E & VROLIJKS, J. 2007. Effects of an African weaver ant, *Oecophylla longinoda*, in controlling mango fruit flies (Diptera: Tephritidae) in Benin. *Journal of Economic Entomology* 100: 695-701.

VAN WYK, B. & VAN WYK, P. 1997. *Field guide to trees of Southern Africa*. Struik Nature, Cape Town, South Africa.

VARGAS, R.I, PIÑERO, J. C, MAU, R.F.L., JANG, E B., KLUNGNESS, L.M., MCINNIS, D.O., HARRIS E.B., MCQUATE, G.T. BAUTISTA, R.C. & WONG, L. 2010. Area-wide suppression of the Mediterranean fruit fly, *Ceratitis capitata*, and the Oriental Fruit Fly, *Bactrocera dorsalis*, in Kamuela, Hawaii. *Journal of Insect Science* 10: 1-17.

VARGAS, R.I., LEBLANC, L., PUTOA, R. & EITAM, A. 2007. Impact of Introduction of *Bactrocera dorsalis* (Diptera: Tephritidae) and Classical Biological Control Releases of *Fopius arisanus* (Hymenoptera: Braconidae) on Economically Important Fruit Flies in French Polynesia. *Journal of Economic Entomology* 100: 670-679.

VAYSSIÈRES, J., GOERGEN, G., LOKOSSOU, O., DOSSA, P. & AKPONON, C. 2005. A new *Bactrocera* species in Benin among mango fruit fly (Diptera: Tephritidae) species. *Fruits* 60: 371-377.

VAYSSIÈRES, J., KORIE. S. & AYEGNON, D. 2009a. Correlation of fruit fly (Diptera Tephritidae) infestation of major mango cultivars in Borgou (Benin) with abiotic and biotic factors and assessment of damage. Crop Protection 28: 477–488.

VAYSSIÈRES, J.-F., KORIE, S., COULIBALY, O., TEMPLE, L. & BOUEYI, S. 2008. The mango tree in northern Benin (1): cultivar inventory, yield assessment, early infested stages of mangoes and economic loss due to the fruit fly (Diptera: Tephritidae). *Fruits* 63: 335-348.

VAYSSIÈRES, J-F., SINZOGAN, A., KORIE, S., OUAGOUSSOUNON, I. & THOMAS-ODJO, A. 2009b. Effectiveness of Spinosad Bait Sprays (GF-120) in controlling mango-infesting fruit flies (Diptera: Tephritidae) in Benin. *Journal of Economic Entomology* 102:515-521.

VERGHESE, A., TANDON, P.L. & STONEHOUSE, J.M. 2004. Economic evaluation of the integrated management of the oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae) in mango in India. *Crop Protection* 23: 61–63.

WAAGE, J.K. & HASSELL, M.P. 1982. Parasitoids as biological control agents – a fundamental approach. *Parasitology* 84: 241-268.

WARE, A.B., DU TOIT, C.L.N., MOHAMED, S.A., NDERITU, P.W. & EKASI, S. 2012. Cold tolerance and disinfestation of *Bactrocera invadens* (Diptera: Tephritidae) in 'Hass' Avocado. *Journal of. Economic. Entomology* 105: 1963-1970

WHITE, I.M. 2006. Taxonomy of the Dacina (Diptera: Tephritidae) of Africa and the Middle East. *African Entomology* Memoir No. 2: 156.

WHITE, I.M., ELSON-HARRIS, M.M. 1994. *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International, Wallingford, UK.

WILLIAMS, V.L., RAIMONDO, D., CROUCH, N.R., CUNNINGHAM, A.B., SCOTT-SHAW, C.R., LÖTTER, M. & NGWENYA, A.M. 2008. *Harpephyllum caffrum* Bernh. ex Krauss. National assessment: Red list of South African plants version 2011.1. Available at: http://redlist.sanbi.org/species.php?species=869-1. Date of access: 16 November 2011.

WILSON, L. G., BOYETTE, M. D., & ESTES, E. A. 1999. Postharvest handling and cooling of fresh fruits, vegetables, and flowers for small farms. Part I: *Quality Maintenance. North Carolina State University.*

WTO.1995. *Agreement on the application of sanitary and phytosanitary measures.* World Trade Organisation, Geneva. Available at: http://www.wto.org/english/tratop_e/sps_e/spsagr_e.htm. Date of access: 12 March 2011.