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SELECTIVE CONTROL OF *OROBANCHE RAMOSA* IN POTATO WITH SUB-LETHAL DOSES OF GLYPHOSATE AND OTHER SOIL TREATMENTS

By

ELIE TONY SHDEED

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By
ELIE TONY SHDEED

Approved by:

M. Haidar

Mustapha Haidar, Professor — Advisor
Agricultural Sciences

Isam Bashour

Isam Bashour, Professor — Member of Committee
Agricultural Sciences

Adib T. Saad

Adib Saad, Professor — Member of Committee
Agricultural Sciences

Salwa Tawk

Salwa Tawk, Research Associate — Member of Committee
Agricultural Sciences

Date of thesis defense: October 25, 2013

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AN ABSTRACT OF THE THESIS OF

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Title: Selective control of *Orobanche ramosa* in potato with sub-lethal doses of glyphosate and other soil treatments

Greenhouse and field experiments were conducted during 2009-2010 at the greenhouse area of the Faculty of Agriculture and Food Sciences (FAFS) and Agriculture and Research Center (AREC) of the American University of Beirut, to test for methyl bromide alternatives such as ammonia gas, phosphoric acid and sulfuric acid for the *Orobanche ramosa* control and to examine *Orobanche ramosa* control with sub-lethal doses of glyphosate and on the yield and quality of potatoes such as size, knobbiness and cracking of tubers. Data were collected on *Orobanche* shoot number and dry weight, potato plant height, vigor, tuber number and marketable and non-marketable yield. Results regarding *Orobanche* total infestation showed that (unlike methyl bromide), the use of phosphoric acid, sulfuric acid and ammonia gas had no significant effect on the level of *Orobanche* infestation as compared to the control. The best results considering both *Orobanche* control and selectivity in potato was obtained by sub-lethal doses of glyphosate. At all tested rates, glyphosate significantly reduced *Orobanche* infestation as compared to the control. However, the increase in the glyphosate rate decreased the number of marketable potato tubers.

Keywords: Orobanche ramosa, glyphosate, potato, soil amendments

Content

page

ILLUSTRATIONS

TABLES

Table Page

ABBREVIATIONS

^{o}C	Degrees Celsius
%	Percent of a Hundred
ai	Active Ingredient
AUB	American University of Beirut
AREC	Agricultural and Research Education Center
ANOVA	Analysis of Variance
cm	Centimeter
CO_2	Carbon Dioxide
DAPE	Days After Potato Emergence
ha	Hectare
H_2SO_4	Sulfuric Acid
H_3PO_3	Phosphoric Acid
ICARDA	International Center for Agricultural Research in the Dry Areas
g/ha	Gram per Hectare
K	Potassium
Kg	Kilogram
Kpa	Kilo Pascal
L/ha	Liter per Hectare
LSD	Least Significant Difference
m^2	Meter Square
N	Nitrogen
P	Phosphorous
SAR	Systemic Acquired Resistance
spp.	Several Species
t/ha	Ton per Hectare
®	Registered Trademark

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum*) is considered one of the most important crops in the East Mediterranean regions and the fourth major world food crop after corn, rice and wheat. In Lebanon, the Beq'aa and Akkar provinces are the main potato production areas in the country, with about 68% and 19% of the total production, respectively (Abou-Jawdah *et al.*, 2001). Potato is susceptible to several pests like viruses, fungi, bacteria, phytoplasma, nematodes and *Orobanche*, some of which are quite difficult to manage and therefore are placed under either quarantine or certification regulations. However, it is one of the most susceptible hosts to *Orobanche ramosa* which parasitizes summer, early spring and autumn planted potatoes across Lebanon and the Mediterranean region.

Parasitic plants account for nearly 1% of angiosperm species and are present in 22 botanical families (Goldwasser and Kleifeld, 2004). Four main families of parasitic plants are agriculturally important parasitic weeds; Orobanchaceae family is one of them. Parasitic angiosperms are generally separated into holo- and hemiparasites. Hemiparasites contain some chlorophyll and gain mineral nutrients and water by connecting to the host xylem via the haustorium. Witchweeds (*Striga* spp.) is an important hemiparasite that has a great impact on wide range of crops grown widely in Africa and Asia (Spallek *et al.*, 2013). However, holoparasites lack chlorophyll and rely totally on the contents of the host xylem and phloem. Although the differences between the two imply absolute and discrete categories, some parasitic plants are intermediate between the hemi- and holoparasitic condition, e.g. *Cuscuta* (dodder). The Orobanchaceae (Known as Broomrapes) are obligate root holoparasite that attack roots of certain dicotyledous plants (Joel *et al*., 2007). Some species are specialized in parasitizing a few groups of plants like *Orobanche crenata* on legumes and *Orobanche cumana* on sunflower (*Helianthus annuus*). In contrast, others species have a broad spectrum of hosts as it is the case of *Orobanche aegyptiaca* and *Orobanche ramosa* (Parker and Riches, 1993; Press and Graves, 1995).

Orobanche species are aggressive parasitic weeds that have a tremendous impact on agricultural crops in the Mediterranean and the warm temperate areas of Europe, North Africa and the East Asia (Joel *et al*., 2013). This problem has been aggravated by the increase in potato cultivation. Increased population pressure in Lebanon, Syria and North Africa has increased the production of the most strategic crops such as legumes and potato, which in turn are *Orobanche* hosts. Accordingly, the extent and intensity of *Orobanche ramosa* infestation has escalated and currently threatens potato production in Lebanon (Haidar *et al.*, 1995).

Despite the availability of practices to slightly control broomrapes in economically important crops, *Orobanche ramosa* infestation continues to increase, threatening the livelihoods of millions of farmers, especially poor ones. Most of the management technologies have not been disseminated to farmers who continue to use ineffective control practices that worsen the problem. The adaptation and dissemination of appropriate control practices are major concern in *Orobanche* control. However, such work requires a community-based integrated management approach because *Orobanche* is a considered community threat.

One of the most promising methods for managing *Orobanche* infestation is the use of sub-lethal doses of glyphosate. In our study, three experiments were held; one in the greenhouse area, the other two in the open field. Several rates and sequential applications of sub-lethal doses of glyphosate were investigated and compared to other soil treatments for the control of *Orobanche ramosa* in potato.

CHAPTER II

LITERATURE REVIEW

A. Parasitic Plants (Description)

Parasitism is an extremely successful life scheme that bridges all kingdoms of life (Poulin and Morand, 2000). Parasitic weeds have a wide environmental range and are represented by about 4100 species in around 19 families (Nickrent and Musselman 2004; Press and Phoenix, 2005). They are divided into two main groups depending on the availability of chlorophyll. Holoparasites lack chlorophyll and depend completely on their host for assimilates such as the families of Lennoaceae, Orobanchaceae and Hydrnoraceae. Hemiparasites contain little chlorophyll and can perform photosynthesis to some extent. Some of hemiparasites can survive either as a parasite or on their own roots, and these are called facultative parasites (Joel *et al.*, 1995). Parasitic *Striga* spp has photosynthetic leaves and thus belongs to the hemiparasites. Parasitic weeds are characterized by the ability to feed directly on host plants, invading several different organs (Stems or roots) through parasitic structures called haustoria. *Orobanche* spp. for example, attach to the roots of their hosts. The parasite is rootless and leafless and considered obligate root parasites (Holoparasite) that obtain all the assimilates they need from the host (Musselman, 1980). Photosynthesis is very low and cannot support *Orobanche* growth and development due to deletions and rearrangements in the chloroplast genome (dePamphilis and Palmer, 1990).

B. *Orobanche* spp. Biology and Distribution

Orobanche or Phelipanche (Broomrape) is an obligate root parasite in the family Orobanchaceae. It attacks roots of various dicotyledonous plants (Joel *et al.*, 2007). This family includes 90 genera and around 1800 species of all the families of phanergamic plants (Nickrent and Musselman, 2004). *Orobanche* spp have non-photosynthetic fleshy stems and therefore belong to the holoparasites. *Orobanche ramosa, Orobanche crenata* and *Orobanche aegyptiaca* are among the most important economic species (Fig. 1). The genus *Orobanche* is mostly widespread in the semi-arid regions of the world (Saghir *et al.*, 1973). According to Musselman (1991), *Orobanche ramosa* arises as a weed in native vegetation in crops in the Mediterranean region and South west Asia and in Italy and Greece and further north in cultivated crops and gardens (Chater and Webb, 1972). It has been reported by Musselman and Nixon (1981) that the *Orobanche ramosa* was first discovered in Central Texas attacking several dicot families of high economic importance.

Figure 1.Photos of different *Orobanche*species.From left to right, O. *ramosa, O. crenata* and *O. aegyptiaca.*

C. Host Range

Although there are over 100 *Orobanche* spp. that mainly attack broadleaf crops, only *Orobanche crenata*, *Orobanche ramosa*, *Orobanche cumana* and *Orobanche aegyptiaca* parasitize agronomic crops (Bouwmeester *et al.*, 2003) such as legumes (bean, chickpea), solanaceous crops (eggplant, tomato, tobacco, potato, peppers), umbelliferous crops (carrot, parsley, celery), brassicaceae (cabbage, lettuce and cauliflower) and sunflower (Joel, 2000; Nickrent and Musselman, 2004; Press *et al.,* 2001). However, each of the *Orobanche* species accumulated several specific host preferences, and the range of the host to attack is influenced by several factors involved in the *Orobanche*'s life cycle. *Orobanche ramosa* is the most epidemic parasite, it can parasitize plants from eleven different dicot families, and these include hosts of great economic importance such as cabbage, rapeseed, tomato, cauliflower, hemp, carrots, lettuce and some legumes (Nickrent and Musselman, 2004). In Lebanon, *Orobanche ramosa* parasitizes mainly solanaceae crops such as potato and tomato. High levels of *Orobanche* infestations caused severe decrease in potato yield quality and quantity (Haidar and Bibi, 1995).

Field surveys conducted in Lebanon by Haidar and Bibi in 1995 and in the West Bank and Gaza Strip by Musselman *et al.*, in 1989 showed that the *Orobanche* species are the dominant and the most widespread parasitic weeds that attack economic value crops such as solanaceous and leguminous crops. In Lebanon, it has been reported that the following plants are host of *Orobanche* spp (Table 1) (Haidar oral communication). In addition *Orobanche* found to parasitize the following weeds: *Galium tricone*, *Malva parvifloraspp*, *Oxalis coniculata*, S*olanum nigrum* (Abu-Irmaileh, 1979) and *Lamium* spp (Haidar, oral communication)

Table 1. Major plants parasitized by *Orobanche* spp.

Family / Crop	**Crop**	***O. ramosa***	***O. aegyptiaca***	***O. crenata***
Compositae	Safflower	X	X	---
Cruciferae	Cabbage	X	X	---
Cucurbitaceae	Cucumber	X	X	---
Iridaceae	Saffron	X	X	---
Leguminosae	Faba bean Alfalfa Pea	--- X X	--- X X	XXX --- X
Rosaceae	Almond	X	---	---

Solanaceae	Egg plant Potato Tobacco Tomato	XX XXX XXX XXX	XX XXX XXX XXX	--- --- --- X
Umbelliferae	Carrot Parsley Lettuce	X X X	X X ---	X ---

XXX = Seriously attacked ; XX = Moderately attacked ; X = Lightly attacked ; --- = Attack doubtful

D. Economic Impact

Parasitism by *Orobanche* has a major consequences on host plants and is common in warm and dry areas, such as the Middle East, India and large parts of Europe and North America (Joel *et al*,. 1995; Press *et al.*, 2001; Verkleij and Kuiper, 2000), with yield losses ranging up to complete crop failure depending on the level of infestation (Foy *et al.,* 1989). It has been estimated that the *Orobanche* spp. affect more than one million hectare of arable land (Verkleij and Kuiper, 2000). In Southern Europe and the Middle East, the general distribution and incidence of *Orobanche* species have been reported in Morocco by Schmitt (1978) in Southern Spain (Mesa-García*et al.*, 1984b) and Syria (Sauerborn and Saxena,1987). In a study of a cultivated area in Morocco, *Orobanche crenata* was found in 78% of the areas studied, totally decreasing the yield up to 20%. In Egypt, 33% in yield loss was reported by Kadry *et al.* (1959), whereas in Turkey the loss ranged from 30-70% (Moiseeva *et al.*, 1969). In Andalucía, Southern Spain, *Orobanche crenata* was reported in 85% of the studied fields, causing severe damages in about 20% of them. However, farmers used to delay the planting of faba bean in their rotation in order to avoid the emergence of *Orobanche* plants, thus, decreasing the economic losses and limiting the area of this crop. In Syria, *Orobanche crenata* was found in 71% of the faba bean fields in the North East region of Aleppo (Manschadi *et al.*, 2001) and reported a yield loss of 5-24% (ICARDA, 1985).

E. Life Cycle and Chemical Signaling Between Parasite and Host

Orobanche reproduces by producing thousands of minute seeds that can remain viable in the soil for many years. It was reported that *Orobanche ramosa* seeds can retain their viability when stored under laboratory condition for up to 20 years (Gold *et al.*, 1987). The life cycle of *Orobanche* is regulated or affected by signaling molecules that are exchanged between the parasite and its host (Fig. 2). The germination chemical stimulants are secreted by the host root and trigger the germination of *Orobanche* seeds (Bouwmeester *et al.*, 2003; Press and Graves 1995). However, seeds will not respond to this signal unless they are subjected to a conditioning period (Pre-treatment) of ten to fourteen days under suitable temperatures (usually between 21 and 30˚C) and moist conditions, a treatment known as pre conditioning (Matusova *et al.*, 2004).

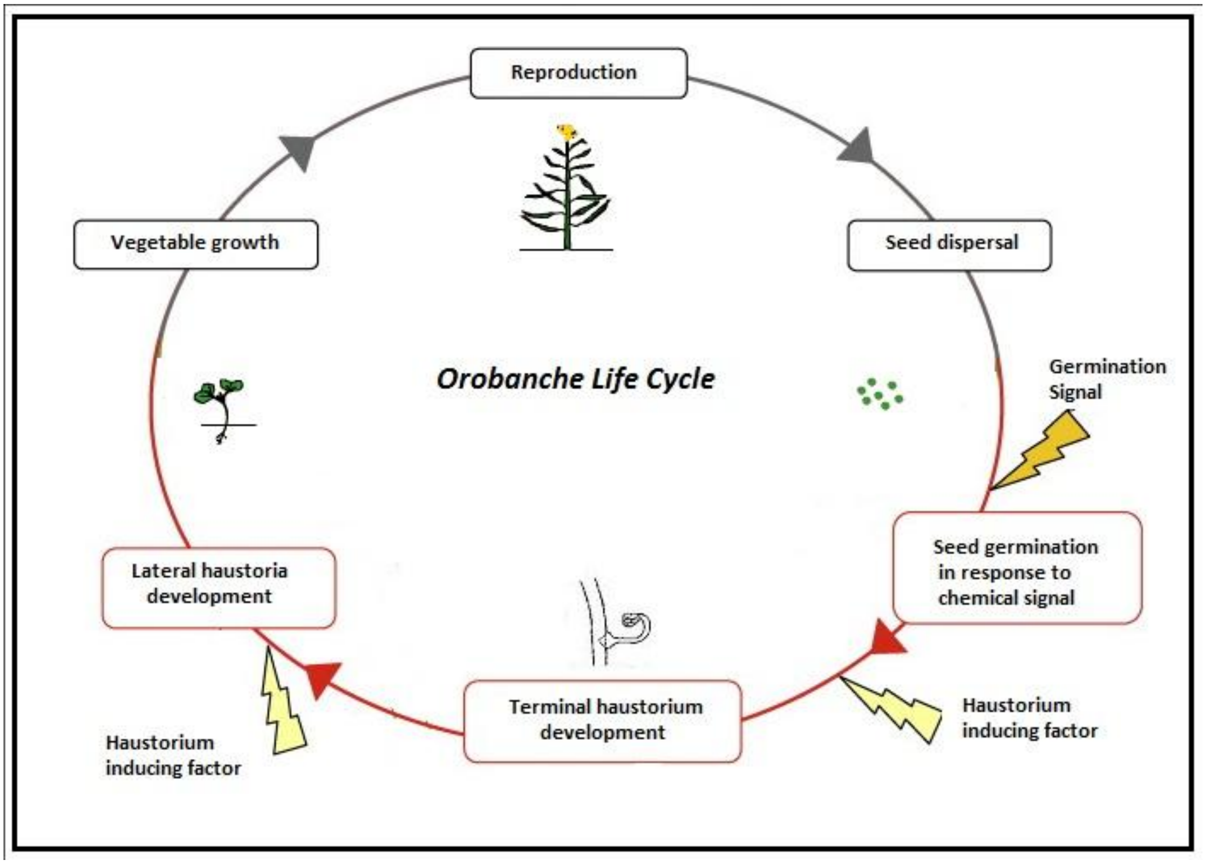

Figure 2. Life cycle and haustorium morphology of parasitic *Orobanche*. Underground, the obligate parasite *Orobanche* requires a host-derived germination signal for the seeds to germinate and produce a terminal haustorium that must connect to the host vascular tissue before further plant development can proceed. *Orobanche* has limited root systems; the roots are capable of forming lateral haustoria upon encountering a host root or a haustorium-inducing factor.

When these conditions are met, respiration of the dormant seeds initiates and apparently induces general metabolic activity. A study by Bar Nun and Mayer (1993) showed that protein synthesis is highly active during the early phases of preconditioning.

However, conditioning makes the seed sensitive to germination stimulants, which consequently induce germination. The chemical stimuli that initiate germination and host detection are called germination stimulants (Fig. 3). They are secreted by the host root and trigger the germination of the parasites seeds (Bouwmeester *et al.,* 2003; Press and Graves, 1995). For example, alectrol and orobanchol, the germination stimulants of *Orobanche minor*, were isolated from its host (*Trifolium pratense* L.) root exudates (Yokota *et al.*, 1998). These plant products were unlikely synthesized and exuded for this purpose, rather they are considered as a part of a defense mechanism. The co-evolution of the host plant and its parasite had led to a situation in which the parasite takes advantage of these exudates to recognize a viable host by which they could complete their life cycle. Currently, few germination stimulants have been isolated and characterized as inductors from natural hosts; all of them belong to plants parasitized by members of the *Orobanchaceae* and *Striga* families (Galindo, 2004). All of these exudates belong to the same skeletal type, named strigolactones (Fig. 3). These compounds share a common backbone and present a lactone-enol-γ-lactone that has been identified as the bioactiphore of the molecule. The mechanism of attachment of these molecules has been proposed by Mangnus and Zwanenburg (1992). However, other natural compounds and synthetic derivatives have been also found to induce *Orobanche* or *Striga* germination, but none of them have been isolated from their typical host. The ability of *Orobanche* to respond to these germination stimulants is of evolutionary significance: their minute seeds contain limited reserves and the seedlings will die within a number of days after germination unless a host root is detected (Butler, 1995). When germination starts, the radicle must detect and grow towards the host root (Fig. 2). This process is possibly directed by the chemical concentration gradients (Dube and Olivier, 2001). Upon attachment to the host root the redicle develops a connective organ known as the haustorium. The haustorium penetrates the root by enzymatic activity and connects the vascular system of the host with that of *Orobanche*. A nodule will be formed following the connection with host and *Orobanche* will develop a so-called tubercle within 10days that helps to accumulate nutrients and eventually a flowering shoot emerges above the soil flowers and set seeds (Kroschel, 2001). However, *Orobanche* spp have a long underground phase (hypogeal, up to 65 days), so that when they appear on the surface (Epigeal) most of the damage to the host has already occurred (Singh *et al.*, 1972).

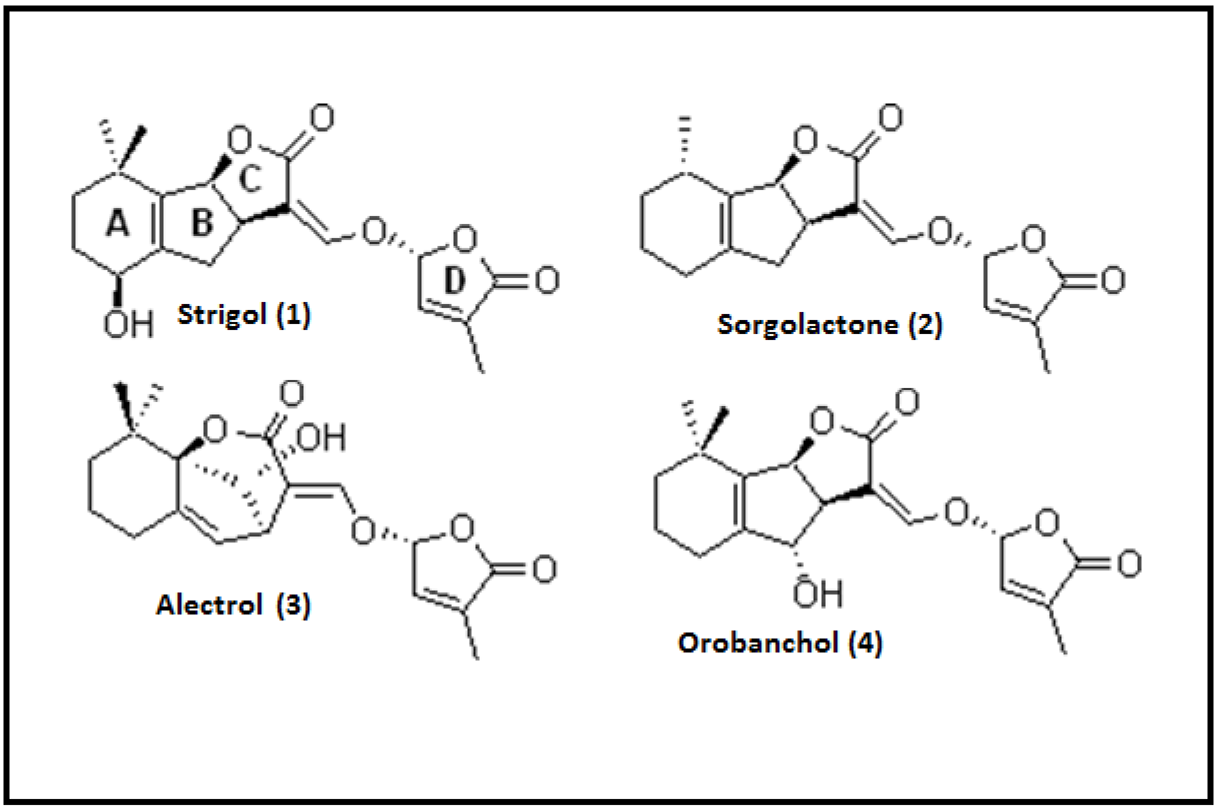

Figure 3.*Striga* and *Orobanche* germination inductors isolated from natural hosts. Rings C and D are the lactone-enol-γ-lactone moiety

F. Host-Parasite Relationship

The survival of *Orobanche* fully depends on its ability to detect and attach to a host plant. Therefore, *Orobanche* evolved survival mechanisms that enable it to recognize host exuded chemical signals to ensure that the roots of the host are in close vicinity. On the other hand, there are several published articles indicate that the composition of root exudates plays a key role in detecting host specificity during the germination phase. For example, *Striga* seeds respond differentially to the germination stimulants from the root exudates of maize (host), cowpea (non host) and GR24 (Matusova and Bouwmeester, 2006). The complexity of the parasitization process includes the establishment of physical and physiological associations between the *Orobanche* and the host plant in order to draw resources and to initiate defense responses in the host (Joel, 1998; Westwood *et al.*, 1998). The *Orobanche* affects the host by altering source-sink relationships that will result in modification in the host resources allocation and root-shoot ratios (Graves, 1995). However, little is currently known about the dynamics of this interaction. Anatomical and physiological methods have been applied to understand the parasite influence on its hosts and to characterize cases of host resistance especially in *Orobanche* (Cubero, 1991). More tractable methods that facilitate molecular, genetic, and mutational approaches are needed to analyze and dissect the parasite–host interaction.

G. Control Measures

Several management strategies have been tested against *Orobanche*, these include hand pulling, fertilizer application, early sowing, the use of trap and catch crops, herbicides, soil amendments and chemical synthetic germination stimulants (Cooke, 2002).

So far these tools have only had a limited impact on controlling *Orobanche*. Currently, there is no single technique that can successfully control *Orobanche* (Haidar *et al.*, 2005). Any management or eradication program must aim at reducing the *Orobanche* production of new seeds and their dispersal to new sites. Control methods that prevent seed germination are expected to be more effective than those affecting later stage of development because they prevent parasitism prior to crop damage and could also reduce the seed bank, for example trap and catch crops (Joel *et al.,* 1995). Hygiene and quarantines are also very essential procedure in the control and eradication programs for *Orobanche*. Individual control techniques of *Orobanche* will be reviewed under chemical and non-chemical measures or strategies.

1. Chemical Control

This part will cover the use of fumigants, other chemicals, herbicides and synthetic stimulants for the control of *Orobanche.*

a. Fumigants

Soil fumigation is the most popular chemical treatment applied against *Orobanche*. It targets *Orobanche* seed bank at the soil surface by using various fumigants like Methyl Bromide. Fumigation by compounds that release methylisothiocyanate was suggested for the control of *Orobanche*. Metham sodium and dazomet applied directly by injection via irrigation systems or incorporated directly to the soil followed by irrigation were found to be very effective for *Orobanche* infestation control (Goldwasser *et al.,* 1995). In all treatments, the soil must be wet at the time of application as seeds are only susceptible when they are in the imbibed state. Another fumigation method with

1,3-dichloropropen applied by injection into the soil followed by sealing and rolling the soil surface or by frequent sprinkler irrigation (Jacobsohn *et al.,* 1991) was effective against *Orobanche crenata* but less potent against *Orobanche aegyptiaca.* Zahran (1970) and Parker and Riches (1993) reported that methyl bromide at various concentrations reduced *Orobanche* infestation by 100%. Later Goldwasser *et al*. (2004) reported that soil fumigation with Metham Sodium reduced *Orobanche* by 50%.

b. Other Chemicals

A promising control tested was the use of ammonium nitrate applied to the soil to inhibit *Orobanche* radical elongation by ammonium ions liberated directly from ammonium or from other nitrogen compounds. Several authors reported the direct toxicity caused by nitrogen fertilizers on the seedling radicle of *Orobanche* and *Striga.* In sorghum, nitrogen reduced the damage of *Striga hermontica* enhancing the host's ability to maintain a favorable osmotic potential (Westwood and Foy, 1999). Nitrogen in ammonium form showed to be more inhibitory than nitrate, however, it was observed that the elongation of the seedling radicle was primarily inhibited by ammonium, rather than the seed germination itself. Another study by Mariam and Suwanketnikom (2004) showed that the effect of nitrogen from organic (chicken, cow, goat manure and inorganic fertilizers such as (NH_4NO_3, $(NH_4)_2SO_4$,NH_2CO NH_2) against tomato plant by *Orobanche ramosa* was effective in reducing parasitism and enhancing growth of tomato plants. This was previously demonstrated by Abu-Irmaileh (1994) who found that the drop in germination and the reduction in length of *Orobanche ramosa* radical were proportional to the ammonium concentration.

The effect of elementary sulphur and chicken manure on *Orobanche ramosa* growth and development in eggplant and potato was investigated by Haidar and Sidahmed (2006). Sulphur alone was ineffective against *Orobanche* growth and infestation in eggplants and potato. For both crops, applying chicken manure with sulphur at all tested rates was effective in reducing *Orobanche* growth and infestation early in the season in comparison with the control.

Applications of superphosphate was tested by Southwood (2006) on subterranean clover (*Trifolium subterraneum* L.) infested with *Orobanche minor*, a reduction in the *Orobanche* population was observed when applying superphosphate which is known as a fertilizer for low phosphate soil.

c. Herbicides

Qasem (1998) evaluated the effect of different soil herbicides on the germination and growth of *Orobanche ramosa* in tomato in glasshouse experiments. Chlorsulfuron, pendimethalin and pronamide effectively controlled the pararsite, with chlorsulfuron gave the best control and the least detrimental effect on tomato plants. At 2.44 g/ha, chlorsulfuron completely prohibited parasite invasion when thoroughly mixed with the soil prior to transplanting. Low doses of this herbicide (0.61 g ai. ha^{-1}) were effective against the parasite and less harmful to tomato plants, although *Orobanche* shoots were observed, infestation was significantly reduced.

Imidazolinone family herbicides are selective acetolactate synthase-inhibiting herbicides that have been found selective for *Orobanche* control. Imazapic and imazethaphyr are two imidazolinone herbicides were used by Jacobsohn *et al.* (2001) as sequential foliar applications to control *Orobanche* on carrot crops. In a greenhouse and field experiments, Goldwasser *et al.* (2001) used split liar applications of low rates of the herbicides imazapic and rimsulfuron to control *Orobanche aegyptiaca* and *Orobanche ramosa* on potato. Three doses of imazapic at 4.5g/ha each, sprayed 2 weeks after potato emergence and re-applied at 2-week intervals, prevented *Orobanche* infestation, but the tuber quality was severely damaged. However, three repeated applications of rimsulfuron at 12.5 or 25g/ha at identical timing to imazapic, selectively controlled the *Orobanche* and were found safe for the potato tuber quality. In another experiment conducted by Haidar *et al.*, (2004), a single application of rimsulfuron followed by a single or consecutive applications of sub-lethal doses of glyphosate at a rate of 100 g ai/ha significantly reduced *Orobanche* infestation after 75 and 90 days after potato emergence as compared to the control.

Many other herbicides were used and showed promising results, treflan 48 EC and Assert 250 EC showed a high efficacy (>80%) in controlling *Orabanche Cumana* in sunflower in Romania. Diphenamid and trifluralin reduced the emergence of *Orobanche* growth at concentration of 10ppm (Saghir and Abishakra, 1971). Absorbic acid was also used as a root dip or foliar spray on tomato, induced the chemical resistance and controlled completely the emergence of *Orobanche* (Bhargava, 1991). Another study on the effect of several herbicides on *Orobanche* by Kasasain and Parker (1971) showed that oryzalin, dichlobenil, chlorthiamid, nitralin and chloramben are promising compounds for the pre-emergence control of *Orobanche aegyptiaca*.

d. Effect of Glyphosate

Glyphosate is a non-selective broad spectrum post-emergence foliar applied herbicide registered for use on many food and non-food crops as well as areas where total herbal control is desired. This herbicide is absorbed through the leaves and is translocated mainly in the symplast to all metabolic active parts of the plant (Nandula *et al.*, 1999). The most common uses include control of broadleaf weeds and grasses in hay/pasture, soybeans, field corn, ornamentals, lawns, turf, forest plantings, greenhouses, rights-of-way.

Glyphosate is generally sold as the isopropylamine salt and applied as a liquid foliar spray. When applied in lower doses, it serves as a plant growth regulator. This unmetabolised substance translocates through the host phloem and becomes concentrated and accumulated in the tissues of the *Orobanche*-host attachment, thus inhibiting *Orobanche* growth prior to its shoot emergence. The attached root *Orobanche* serves as a strong metabolic active part of the host plant, rapidly absorbing the chemical through direct connection with the vascular system of the host root. At the cell level, the mode of action of glyphosate is the inhibition of the enzyme EPSP synthase of the shikimic acid pathway, inhibiting aromatic amino acid synthesis and thus protein synthesis and growth (Amrhein *et al.*, 1980). However, some plants that belong to the Apiaceae and Fabaceae families tolerate low rates of glyphosate; this has been attributed to the decomposition of the herbicide to nontoxic substances (Sharon *et al.*, 1992). This trait has been used for selective control of the *Orobanche* with glyphosate in tolerant crops.

One of the most promising methods for controlling *Orobanche* is the use of sub-lethal doses of glyphosate on crops that show tolerance to glyphosate (Elzein and Kroschel, 2003; Dawson *et al.*, 1994; Parker and Riches, 1993; Jacobsen and Levy, 1986; Haidar *et al.*, 2004). The idea is to apply low rates of glyphosate on the host leaves, so that the glyphosate would move through the host phloem to underground *Orobanche* attachment on the host roots and exert its toxic effect, thus inhibiting *Orobanche* growth prior to its shoot emergence. Low rates of glyphosate were found to be effective against *Orobanche*spp in broad bean (Kasasian, 1973; Schmitt *et al.*, 1979), sunflower (Castejon-Munoz *et al.*, 1990), tomato (Kotoula-Syka and Eleftherohorinos, 1990), carrot and celery (Elzein and Kroschel, 2003), vetch (Nandula *et al.*, 1999), parsley (Hershenhorn *et al*., 2002) and potato (Haidar *et al.*, 2004). Preliminary experiments conducted by Haidar *et al.*, 2004 showed that consecutive applications (three times) of sub-lethal doses of glyphosate were selective in potato and reduced *Orobanche* invasion.

e. Chemical Stimulants

A new approach for the management of parasitic weeds is so called “suicidal germination”, that is, the stimulation of seed germination by the application of a germinating stimulant to the soil in the absence of the host. The parasitic weed will germinate in the absence of the host and die which will result in a reduction in the seed bank in the soil. Some fungal metabolites were tested for the false germination of *Striga* and *Orobanche* parasites. For example, fusicoccin and cotylenol proved to induce seed germination of *Striga hermonthica* and *Orobanche* minor (Yoneyama *et al.*, 1998). Fernández-Aparicio *et al.* (2008) found that fusicoccin derivatives 5 and 6 and Ophiobolin

A could represent a potential herbicide in view of their practical application in the suicidal germination of the parasitic *Orobanche* species. Moreover, Methyl isothiocyanate (Zhelev, 1987) and Nijmegen-1 (Wigchert *et al.,* 1999) are two germination stimulants showed promising results against *Orobanche* spp.

Evidente *et al.,* (2010) isolated three new polyphenols, named peapolyphenols A, B and C, together with an already well-known polyphenol and a chalcone (1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4-hydroxyphenyl)-1-propanone and 1-(2,4-dihydroxyphenyl)-3-(4-methoxyphenyl)propenone) from pea root exudates, interestingly, they were found to strongly stimulate *Orobanche* seed germination.

Recent published articles showed that strigolactones, strigol, sorgolactone, orobanchol, sorgomol and 5-deoxystrigol were screened and showed to induce the germination of *Striga hermonthica* seeds collected from mature plants that parasitized on sorghum (Nomura *et al.*, 2013). In another paper, dehydrocostus lactone (DCL) effectively stimulated the germination of *Orobanche Cumana* seeds on sunflower (Daniel *et al.*, 2011).

2. Non-Chemical Control

Various non-chemical agricultural practices have been used for the control of *Orobanche*. These include the time of planting, catch and trap crops, solarization, resistant varieties grazing, and soil amendments.

a. Time of Planting

The effect of planting date of many crops showed to affect the infestation of many *Orobanche* species (Parker and Riches, 1993; Castejon-Munoz *et al.,* 1993). The effect of sunflower (*Helianthus annuus***)** variety 'Amber' planting time showed to be very effective in reducing the germination of several *Orobanche* species. Temperatures regimes in the range (Day/ night) 29-17/ 21-9 ^{o}C caused the degeneration and death of the parasite (Eizenberg *et al.*, 2003; Eizenberg *et al.*, 2009). Early planting of potato in the Beq'aa plain found to be *Orobanche* free unlike late planting (Haidar, Oral communications)

b. Catch and Trap Crops

Orobanche seed germination can be stimulated by a catch or a trap crop. A catch crop is a host crop that is parasitized and must be destroyed before parasite seed development. A local cultivar of *Brassica campestris* has been used as a catch crop in Nepal, reducing the *Orobanche aegyptiaca* seed bank by around 30% (Acharya *et al.*, 2002). Cultivation of berseem clover (*Trifolium alexandrinum*) was used also to reduce *Orobanche crenata* emergence in faba bean, a reduction by 92% over 3 years and by 98% over 4 years (Al-Menoufi, 1991). Applying a catch crop requires an appropriate choice of host plants and a complete control of these hosts by proper timing of eradication.

A trap crop is a false host crop that stimulates the parasite seed germination but is tolerant to the parasite. In other words, they stimulate the germination of *Orobanche* seeds but do not attacked themselves by parasite. Abu-Irmaileh (1984) found that flax (*Linum usitatissimum* L.) and mung bean (*Phaseolus aureus*) can serve as a trap crop for the *Orobanche ramosa.* Pepper (*Capsium annum*) was also used as a trap crop for the control of *Orobanche aegyptiaca* and *Orobanche cernua in* tomato cultivars (Hershenhorn *et al.*, 1996). Sauerborn (1991) suggested that two or three consecutive catch crops grown in the same year are more effective than a single trap crop.

c. Solarization

Soil solarization is another non-chemical practice used for the control for *Orobanche* management. Black mulch, or more usually a polyethylene clear film is used to trap the heat from sunlight. Temperatures of 48-57˚C could be reached and kill *Orobanche* seeds that are on the soil surface (Jacobsohn *et al.*, 1980; Sauerborn *et al.*, 1989; Haidar and Sidahmed, 2000). It was shown that the seeds of *Orobanche ramosa* can survive up to 35 days at 50˚C in dry soil, but are quickly eradicated by temperatures of 40˚C in wet soil (Drennanand Mohamed-Ahmed, 1992). Solarization showed to be more effective if nitrogen fertilizers or chicken manure is added; this can significantly improve the control of *Orobanche* seeds at greater depths (Haidar and Sidahmed, 2000). Due to the high temperatures in the summer period this technique is very effective in the Middle East regions with an endemic *Orobanche* problem.

d. Resistant Crop Varieties

The potential of a host plant to prevent the *Orobanche* functional attachment is referred to be a resistance trait. The degree of resistance varies within the host species and may be influenced by several factors, both heritable and non-heritable. Serghini *et al.*, (2001) showed that *Orobanche cernua* radicles and the host-tissue around the contact point turned brown when the attachment is formed with resistant varieties of sunflower roots. These results suggested the possible accumulation of toxic compounds as a part of the defense mechanism in the resistant sunflower varieties.

Resistance in vetch (*Vicia* spp.) is determined by anatomical characters that provide mechanical resistance to penetration by the *Orobanche aegyptiaca* haustoria, and possibly also chemical characters (Goldwasser *et al.*, 2000). A study by Zehhar and coworkers (2003) on resistant carrot varieties, 'Palaiseau' and ‘Buror’, the *Orobanche ramosa* germinated and attached to the host root but became necrotic before emergence. However, in 'Buror' carrot, the formation of a mechanical barrier was associated with the restriction to the cortex of the parasite.

Sl-ORT1 is a newly fast-neutron-mutagenized M-82 tomato mutant that was shown to be resistant to high concentrations of *Phelipanche aegyptiaca* seeds, and to another three broomrape species: *Phelipanche ramosa*, *Orobanche cernua*, and *Orobanche crenata* (Dor *et al.*, 2010).

Some resistance traits and varieties were found in chickpea and pea, giving new hopes of genetic advancement in these crops (Rubiales *et al.* 2003, 2004). In Egypt, breeders have selected several faba bean varieties that are resistant to *Orobanche crenata*, including Giza429, Giza674, Giza843, Misr1 and Misr2 (El-Shirbini and Mamdouh, 2004). In field experiments performed in Tunisia, the promising response behavior of the breeding line XBJ90 to *Orobanche foetida* was recently reported (Abbes *et al.*, 2007). Its lower susceptibility to the parasite infestation was due to a decrease in the number of *Orobanche* attachments that will lead to the reduction in parasite emergence, however, no parasite necrosis was observed on the host roots.

e. Soil Amendments

Crop residues like wheat and barley showed a promising effect against *Orobanche ramosa.* The effect of wheat and barley residues was studied by Haidar *et al.* (1995) on *Orobanche ramosa* in potato at different application rates and placement positions, it was found that barley straw at 12000 $Kg.ha^{-1}$ significantly reduced the *Orobanche* infestation. Another study by Haidar and coworkers (2003) using broiler, cattle, goat, layer, sheep manure and wood chips as organic soil amendments revealed that goat manure was the only treatment that significantly reduced *Orobanche* infestation in potato throughout the growing season and shoot dry weight (58.5% reduction). All other treatments significantly reduced *Orobanche* infestation only early in the season.

Ghosheh *et al.* (1999) and Altieri *et al.* (2010) showed that amendment of soil with crushed olive pulps (Jift) significantly reduced either the germination or the attachment of *Orobanche* to the host plants.

f. Biological Control

Biological control could be considered in the future as the most promising system to control *Orobanche*. Two seed/stem feeders are entomofauna associated with the *Orobanche* spp. the fly *Phytomyza orobanchia* and the weevil *Smicronyxcyaneus*, are the most promising candidates for weed control. The effect of the insects to control the *Orobanche* can be improved when used in combination with other pathogens such as fungi. Cristofaro (2002) recommended the use of the insect along with bait traps associated with selected fungi spores. Moreover, many toxigenic fungi are able to produce phytotoxins; most of them belong to the *Fusarium* genera. Compounds such as fusaric acid, fumonisins, enniatin, moniliformin and trichothecenes possess a variety of biological activities that causes necrosis, chlorosis, growth inhibition, wilting, and inhibition of seed germination. These bioactive compounds are of different biological and chemical characteristics that could be of great help in integrated management of parasitic plants.

A novel chemical control approach has been developed during the recently called the systemic acquired resistance (SAR). This mechanism is induced in plants by the application of specific chemical agents or with a microorganism. In recent experimental studies, SAR in the host plants has shown of be used for the control of important *Orobanche* species that attack important crops (Sauerborn *et al.*, 2002; Pèrez-de-Luque *et al.*, 2004a; Gonsior *et al.*, 2004; Buschmann *et al.*, 2005).

SAR is a molecular and biochemical response of the plant that triggers unspecific defense responses to various chemicals and to different bacterial, fungal and viral infections (Stichler *et al.*, 1997). The effect of SAR on the *Orobanche*-host systems is still in its initial research stages. A study by Buschmann and Sauerborn (2002) on the application of a resistance inducing agent BION 1 (1,2,3-benzothiadiazole-7-car-bothioic acid S-methyl ester, Syngenta) stimulated the initiation of the defense system in sunflower roots, which protects against parasitism of *Orobanche cuman.* Another field study by Perez-de-Luque (2004) on faba bean had shown that a significant reduction in the population of *Orobanche crenata* upon foliar application of benzothiadiazole BTH. This study achieved highly variable success rates of *Orobanche* management after several modes of application of BTH and salicylic rates on pea in growth chamber experiments. Another plant activator acibenzolar-S-methyl (ASM) reported by Müller-Ströver et al (2005) showed to activate a systemic acquired resistance (SAR) against *Orobanche* *cumana.* Moreover, a recent experiment on the effects of prohexadione-calcium (PHDC) on *Orobanche cumana* showed that this activator induces SAR in sunflower plants and causes a retarded *Orobanche cumana* tubercle formation and development (Fan *et al.,* 2007).

Mabrouk *et al.* (2007a,b,c) used some strains of *Rhizobium leguminosarum* that may release unknown compounds which in turn induce a systemic resistance against *Orobanche crenata* infections in peas. These promising results show that the host-parasite specific application techniques of resistance inducing agents are important to increase the efficiency and reduce the detrimental effects of *Orobanche* infestation.

Individual techniques applied are not totally effective to achieve this reduction, therefore, integrated control using several techniques is highly recommended. Kebreab and Murdoch (2001) constructed a simulation model which predicted that the sustainable control of *Orobanche* requires a permanent reduction in the number of seeds to below 2000 seeds per square meter. However, Linke and Saxena (1991b) suggested a combination of solarization, herbicides and manual weeding with careful selection of cultivars and sowing times to control *Orobanche* in legume crops; none of these methods gave complete control when used separately. Control strategies can only be effective when enough is known about the biology of the *Orobanche* in order to recognize its own life cycle, and the vulnerable points in this strategy.

It may be concluded that the use of strigolactones, sublethal doses of glyphosate, resistant varieties and biological control are the most promising measures against *Orobanche* spp.

CHAPTER III

MATERIALS AND METHODS

Field experiments were conducted at the Agricultural Research and Education Center (AREC) at the American University of Beirut (AUB) located in the Central Beq'aa plain with an altitude of around 1000 m above sea level at 33° 55'latitude and 36° 04' longitude. The experiments were performed during the growing seasons between April and August of the years 2009 and 2010. The green house experiment was done at the greenhouse area of the Faculty of Agricultural Sciences (FAFS), at the Beirut Coastal area, during September 2009 and April 2010. High quality local Spunta seed potato variety were obtained from the Beq'aa local market and used in the three experiments. *Orobanche ramosa* seeds used in all experiments were collected in 2008 from various potato fields in the Beq'aa plain and stored at room temperature until used for experimental purposes.

A. Green House Experiment (Boxes)

This experiment was carried out in the green house area at AUB between September 2009 and April 2010. Several organic and inorganic chemicals were used to investigate their effect on crop and *Orobanche* growth and development. Potato tubers were spread on paper sheets and kept moist in the lab at room temperature three weeks prior to planting. Tubers were planted in plastic netted boxes 30 x 40 x 30 cm, each contained soil mixture of potting soil (terreau), perlite and peat moss at a rate of 1:1:1. Each box, except for the control (No *Orobanche)*, was inoculated with 100mg of

Orobanche ramosa seeds. Boxes were irrigated with water for a period of two weeks prior to planting potato or applying treatments with the chemicals (Table 2) to allow for conditioning of *Orobanche* seeds. Then two tubers were planted per box.

Table 2.Greenhouse experiment layout

Treatment	**Rate**	**Time of Application**	**Type of application**
Control (No *Orobanche*)			
Control (With *Orobanche*)			
Methyl Bromide	$900g/10m^2$	Before planting	Injection
Glyphosate	125ml ai/ha	20 DAPE	Foliar
		20, 40 DAPE	
		20, 40, 60 DAPE	
	135ml ai/ha	20 DAPE	
		20, 40 DAPE	
		20, 40, 60 DAPE	
	150ml ai/ha	20 DAPE	
		20, 40 DAPE	
		20, 40, 60 DAPE	
H_2SO_4	pH3 (2L/box)	Once/week	Irrigation
H_3PO_4	pH3 (2L/box)	Once/week	Irrigation

DAPE: Days after potato emergence

The experiment was arranged with four replicates. A fertilizer of N-P-K (20-20-20) applied at $20g.box^{-1}$ one time for all boxes at the beginning and then irrigated with water or chemical treatments.

1. Injection with Methyl Bromide

Methyl bromide was injected in the boxes prior to planting potato. Four moist boxes were placed in an area of $10m^2$ covered with nylon sheet and fumigated with Methyl Bromide at a rate of 900g per $10m^2$. Sheets were removed 24hrs after fumigation and boxes placed back in the greenhouse. Potato tubers were planted 20 days after fumigation.

2. Injection with Ammonia Gas

The ammonia gas was used at a rate of 1.4 Kg for 40 Kg soil (which is equivalent to 4 boxes approx.). The boxes were covered with clear nylon sheet and then the ammonia gas was injected inside. Boxes were left covered for 1 week then planted immediately with potato.

3. Application of Glyphosate

Glyphosate was applied as a sequential foliar spray at three different sublethal doses, namely, 0.125, 0.135 and 0.150 $kg.ha^{-1}$ in a spray volume of 1000 L/ha on January 5, 29, February 19, 2010 respectively. Glyphosate was sprayed on the potato foliage 20 (single), 40(twice) and 60 (three times) days after potato emergence. A small Universal spray kit was used for this purpose.

4. Application of Sulfuric Acid or Phosphoric Acid

Each of the sulfuric acid or phosphoric acid solutions was applied directly to the planted potato in boxes once per two weeks. Both acids were diluted in water and irrigated at a volume of one liter per box each two weeks starting 0 DATE.

B. Field Experiment One

This experiment was conducted in a naturally infested field with *Orobanche ramosa* at the Agricultural Research and Education Center (AREC) of the American University of Beirut, Lebanon during April-August 2009 (Fig. 4). The soil was silty clay loam with a pH of 7.41, E.C. 0.24 ms/cm and 2.4 % organic matter. All plots were tilled twice with a mouldboard, disked and levelled two weeks prior to planting. The experimental area received a uniform application of 2.5 $t.ha^{-1}$ of NPK (17:17:17) before disking. Nitrogen was applied 40 days after planting at a rate of 300 kg/ha each. Plots were sprinkler irrigated every eight days. Treatments with glyphosate or ammonia were used to investigate there effect on *Orobanche* control and on potato yield.

Figure 4.Naturally *Orobanche ramosa* infested potato field

Potato cultivar Spunta was planted at 3.0 $t.ha^{-1}$. Potato rows were 0.75 m apart and within-the-row spacing was around 0.35 m. Plots (10m long) (See figure 5) were hilled 6 weeks after planting (standard practice by Lebanese farmers). To eliminate all weeds other than *Orobanche*, the entire experimental area received a standard application of metribuzin (Sencor^R, 70%, PE) at 0.75 kg.ha^{-1} one week after potato sowing.

A split plot design (Fig. 5) was used with 12 rows per plot. Ammonia gas was injected one week prior to potato planting, 4.5, 5 and 6 kg were applied in a 200m^2 block. In other plots, sequential application of foliar sub-lethal doses of glyphosate (Round up^R, 48%) at a rate of 125 g.ha^{-1} was applied at 40, 60 and 80 (See figure 9) days after potato emergence (DAPE). Glyphosate was applied with a CO_2-pressurized backpack sprayer that delivers 310 L/ha at 138 Kpa through a Teejet 8002 flat fan spray tips.

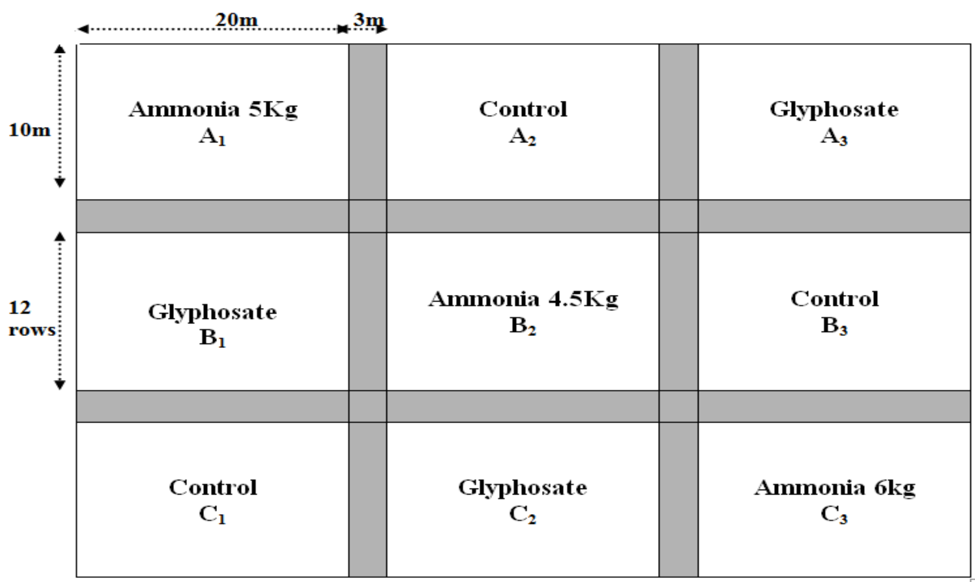

Figure 5. Experimental design of the first field experiment

C. Field Experiment Two

In this experiment, potato tubers were planted in the same field and same operations and management as the first field experiment but at different location in April 2010. A split plot design (Fig. 6) was used with 10 rows per plot, each row had a width of

0.75m and 5m long. Potato tubers sprouted 20 days after planting. Metribuzin was applied 12 after planting potato (PE) at the rate of 0.75 $kg.ha^{-1}$. Urea and fungicides were applied as a band application 50 DAPE. Sequential application of foliar sub-lethal doses of glyphosate (Round up^{R}, 48%) at 60, 80 and 100 $g.ha^{-1}$ applied at 20, 40 and 60 DAPE. The above rates were selected after a preliminary greenhouse study. Four replicates of each treatment were performed. Four plots without spraying were considered as a control (Table 3).

Table 3.Second field experimental design

Treatment	**Rate (g ai/ha)**	**Time of application DAPE**	**Plot No by replicate**			
Control	0	0	101	210	305	403
Glyphosate	60	20	102	209	306	404
	80	20	103	208	301	405
	100	20	104	207	302	406
	60	20/40	105	206	303	407
	80	20/40	106	205	304	410
	100	20/40	107	204	310	408
	60	20/40/60	108	203	307	409
	80	20/40/60	109	202	308	401
	100	20/40/60	110	201	309	402

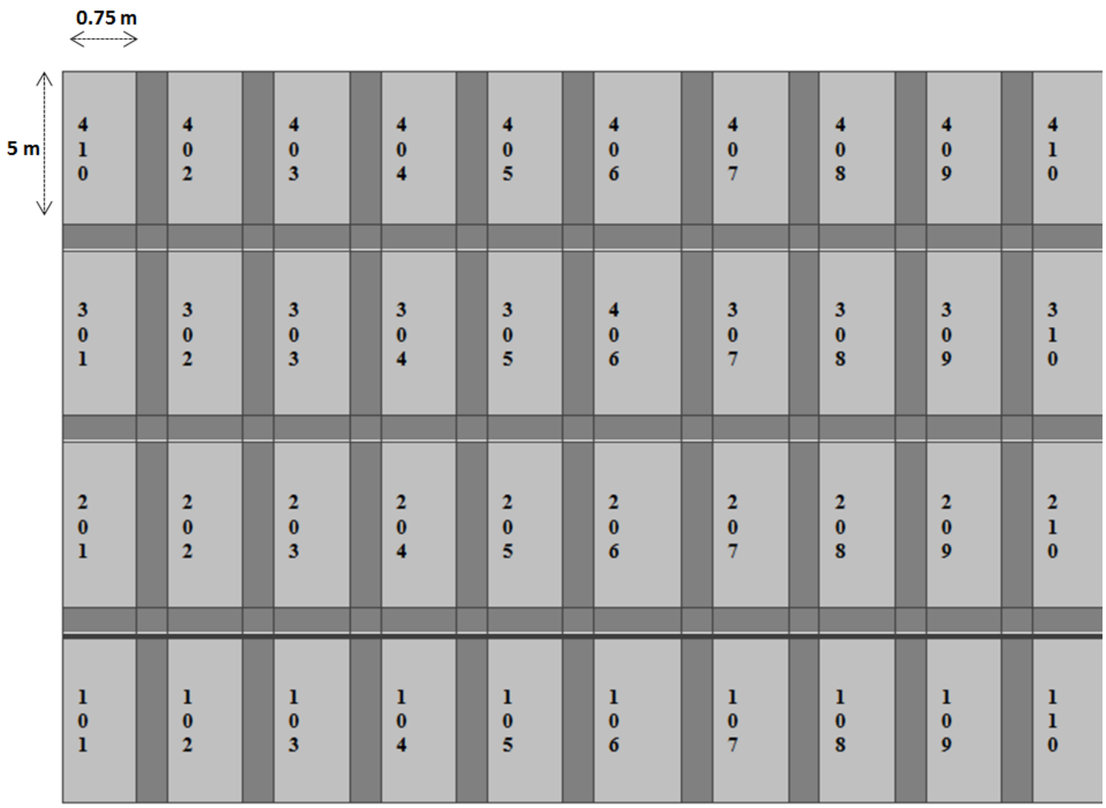

Figure 6.Spraying field map of the second field experiment

D. Experimental Measurements and Statistical Analyses

Orobanche data included shoot number and shoot dry weight per 1 m^2. Potato data included phytotoxicity visual rating taken according to the European Weed Research Council scoring system (Bolle, 1964), number of shoots of the middle row (40 DAPE), average potato height (60 and 75 DAPE, 5 plants per plot), total yield ($t.ha^{-1}$) and tuber number of marketable and non-marketable yield per 3 m^2. Potato yield was determined by harvesting $3m^2$ of the middle row in each plot. Yield quality was determined by separating harvested tubers into two classes: marketable (>5.0 cm in diameter) and non-marketable tubers (<5.0 cm in diameter) according to Robinson *et al.* (1996). Dry weight of

Orobanche shoots were determined by placing them in oven at 70 ºC for 48 hrs. Analysis of variance (ANOVA) were performed through SAS (1992), means were compared by the Student-Newman-Keuls Test at 5% probability.

CHAPTER IV

RESULTS and DISCUSSION

A. Green House Experiment

1. *Orobanche* Management

A single or sequential application of sub-lethal doses of glyphosate at 125, and 150 g ai/ha applied 20, 40 and 60 DAPE (See table 2, page 29) and treatment with methyl bromide significantly reduced *Orobanche* shoot number and shoot dry weight 75 DAPE as compared to the control. All these treatments reduced *Orobanche* infestation by 100% 75 days DAPE. Single application of glyphosate at 135 $g.ha^{-1}$ applied 20 DAPE had no significant effect on shoot number or shoot dry weigh of *Orobanche* after 75 DAPE. However, sequential application of glyphosate at 135 $g.ha^{-1}$ significantly reduced shoot and dry weight of *Orobanche.* Sulfuric acid and phosphoric acid had slightly reduced (Not significant) *Orobanche* shoot number or dry weight in comparison to the control (Table 4).

Using sub-lethal doses of systemic herbicides as single or sequential applications have been found very effective against *Orobanche* in various crops. Split application with low rates of sulfunylurea herbicides inhibited *Orobanche* growth in potato (Goldwasser *et al.*, 2001) and tomato (Hershenhron *et al.*, 1998). Sequential application of sub-lethal doses of glyphosate inhibited *Orobanche* growth in many crops (Elzein and Kroschel, 2003). Haidar *et al.*, 2005 suggested that single foliar application of rimsulfuron at 12.5 $g.ha^{-1}$ followed by sequential foliar application of sub lethal doses of glyphosate at 100 $g.ha^{-1}$ inhibited *Orobanche ramosa* in potato. The significance of using sequential application of systemic herbicides such as glyphosate on potato during the growing season is to inhibit *Orobanche* growth prior to shoot emergence. *Orobanche* seeds are continuously induced to germinate by potato roots and develop the attachment organ, the germ tube or redicle. Sequential application of systemic herbicides may prevent the attachment of the organ or its differentiation and allow for early season control of *Orobanche*.

Injection with methyl bromide was suggested by Wilhelm *et al.*, (1959), who showed good activity of methyl bromide in controlling and eradicating *Orobanche ramosa* in California, it also provided excellent control of *Orobanche* spp. in New Zealand (James, 1976), California (Musselman and Sand, 1982), Greece (Vasilakakis *et al.*, 1988) and Turkey (Nemli *et al.*, 1991). Although methyl bromide is very effective in eradicating and killing *Orobanche*, it is not economical for broad application, and poses significant environmental impacts (Ruzo, 2006).

The little effect of the two acids against *Orobanche* infestation could be due to the small concentrations applied to the boxes, or to the increased rate of irrigation that lead to leaching of the acids. It was previously shown that soil pH affect the seed germination and growth of many plants in soil (Adriano *et al.*, 1973; Simpson, 1986; Zucconi *et al.*, 1981). Haidar and Sidahmed (2005) found that sulfur granules significantly decreased the infestation of *Orobanche ramosa* in potato. Moreover, preconditioning of *Orobanche* seeds with phosphoric acid (21 ml of 1Molar stock solution) showed to decrease the *Orobanche aegyptiaca* germination in tomato (Jain and Foy, 1992).

Table 4. Effect of glyphosate, sulfuric acid, phosphoric acid and methyl bromide on average *Orobanche* shoot number and dry weight (n = 4). Data represent average of four replicates (boxes)

Treatment	Rate	Application time (DAPE)	Shoot number 75		Shoot dry weight (g) 75 DAPE
			Above Ground*	Under Ground	
Glyphosate (g ai/ha)	125	20	0.0 b	0.0 b	0.0 b
	135	20	5.0 ab	4.3 ab	2.0 ab
	150	20	0.0 b	0.0 b	0.0 b
	125	20/40	0.0 b	0.0 b	0.0 b
	135	20/40	0.0 b	0.0 b	0.0 b
	150	20/40	0.0 b	0.0 b	0.0 b
	125	20/40/60	0.0 b	0.0 b	0.0 b
	135	20/40/60	0.0 b	0.0 b	0.0 b
	150	20/40/60	0.0 b	0.0 b	0.0 b
Sulfuric Acid	2L/box		14.8 a	14.8 a	2.6 ab
Phosphoric Acid	2L/box		10.0 a	12.3 a	2.8 ab
Methyl Bromide	900g/10m2		0.0 b	0.0 b	0.0 b
Control without *Orobanche*	0.0		0.0 b	0.0 b	0.0 b
Control with *Orobanche*	0.0		17.8 a	12.3 a	4.6a

*Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.

2. Potato Biomass and Yield

Single or sequential application of glyphosate at all tested rates were toxic to the potato plants and significantly reduced potato shoot height at 30 and 60 DAPE, compared to the control. Sulfuric acid, phosphoric acid, and methyl bromide had no significant effect on potato plants compared to the control (Table 5). Visual potato injury appeared 10 days after the first glyphosate application and included leaf yellowing, necrosis, plant stunting and compact potato shoots. Phytotoxicity was clearly reflected in the tuber quality of potato yield grown for fresh market with a high incidence of deformed (Cracked) and small tubers (Fig. 7 A, B and C). Other treatments with methyl bromide, sulfuric and phosphoric acids did not show any deformation on the potato tuber (Fig. 7D), since the phytotoxicity visual rating of potato was not affected by the treatments.

Table 5. Effect of glyphosate, sulfuric acid, phosphoric acid and methyl bromide on average potato shoot height and vigor (n = 4).

	Rate	**Application time (DAPE)**	**Plant height (cm) DAPE**		**Phytotoxicity visual rating (%) ** 60 DAPE**
			30*	**60**	
Glyphosate (g ai/ha)	125	20	71.3 b	70.8 b	6.5 b
	135	20	66.3 b	78.0 b	6.8 b
	150	20	65.0 b	70.8 b	6.5 b
	125	20/40	71.5 b	76.0 b	7.0 b
	135	20/40	76.8 b	82.8 b	7.5 b
	150	20/40	76.0 b	75.8 b	6.5 b
	125	20/40/60	77.5 b	80.3 b	7.5 b
	135	20/40/60	76.5 b	83.3 b	7.8 b
	150	20/40/60	64.3 b	69.8 c	6.5 b
Sulfuric Acid	2L/box		92.3 a	104.8 a	9.0 a
Phosphoric Acid	2L/box		86.8 a	99.3 a	8.8 a
Methyl Bromide	900g/10 m		83.0 a	92.8 a	9.0 a
Control without *Orobanche*	0.0		90.8 a	102.3 a	8.8 a
Control with *Orobanche*	0.0		87.0 a	90.5 ab	9.0 a

*Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.
**Scale (0–10), with 0 indicating complete death and 10 no injury

Figure 7. A, B, C and D: Deformed potato tubers with cracks or fissures caused by a single application and/or sequential application of glyphosate. D: Normal potato tubers with no significant deformations when sprayed with methyl bromide, sulfuric or phosphoric acids, as well as a positive control.

Methyl bromide, sulfuric and phosphoric acids had no significant effect on the potato yield in comparison with the control (Fig. 8). Applying sub-lethal doses of glyphosate two or three times at a rate of 150 $g.ha^{-1}$ considerably reduced the total yield by 35% and 31%, respectively. All other glyphosate treatments had no significant effect on the potato yield. In turn, the potato tubers were small in size and mostly deformed (Cracked). The non-marketable tuber number significantly increased with the increase of glyphosate rate and in the number of spraying. The highest number of nonmarketable tubers was recorded when spraying with 150 g ai/ha three times. However, all other treatments did not show any significant change in the number of tubers when compared to the control. Even though potato tubers were small and deformed, there was a significant difference in total yield compared to the control from the increase in the number of these deformed tubers. A study by Robinson and Hatterman-Valenti (2013) showed that when glyphosate comes in contact with the potato plant during the growing season, it damaged both the leaves and the tubers, and increased the yield with high percentage of non-marketable potato tubers. It is well known that glyphosate translocates sympoplastically and appoplastically in potato plant. During initial tuber development, tubers accumulate photosynthetic assimilates produced by the leaves and other exogenous compounds such as glyphosate. Thus, the glyphosate effect may appear as yellowing or necrosis in young leaves and malformed tubers.

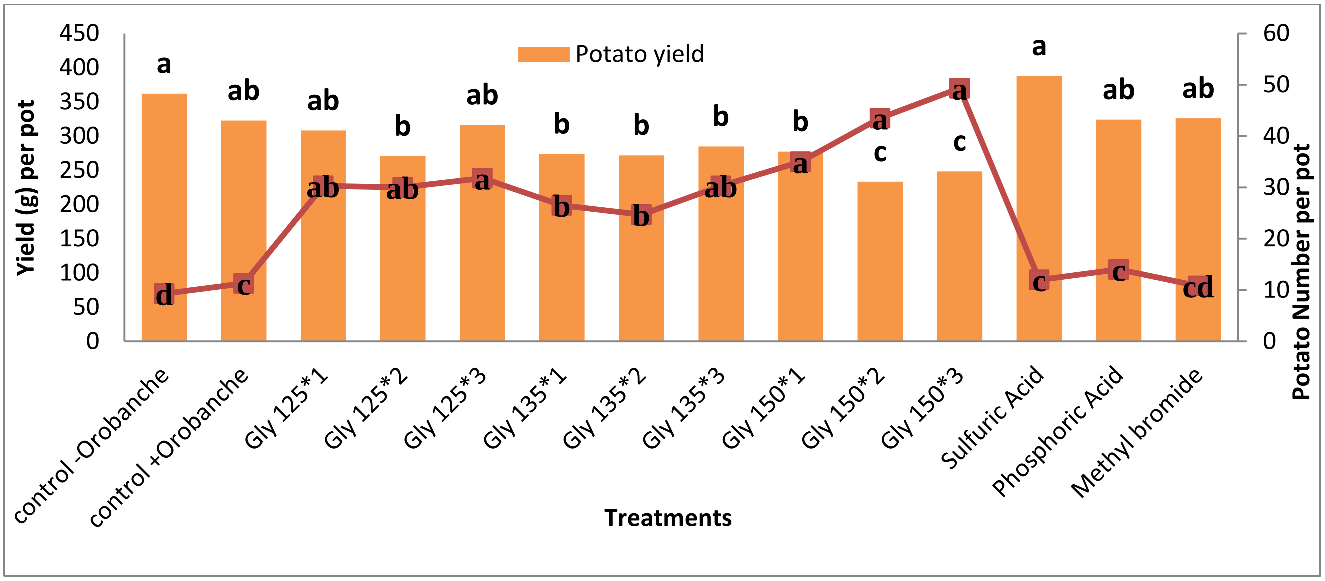

Figure 8. Average potato yield and number of tubers/pot (n = 4) in response to treatments by sub-lethal doses of glyphosate and other soil treatments. Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.

Many researchers reported the destructive effect of low doses of glyphosate on different crops. Pea (Arjona-Berral *et al.*, 1984), sunflower (Castejon-Munoz *et al.*, 1990), tomato (Kotoula-Syka and Eleftherohorinos, 1990) and potato (Haidar *et al.*, 2005) were sensitive to sub lethal doses of glyphosate. Thus, selectivity remains the main obstacle in various host plants and it could be mediated by the time and rate of application of systemic herbicides (Elzein and Kroschel., 2003; Mesa-Garcia and Garcia-Torres, 1985).

B. Field Experiment One

1. Orobanche Management

Data on the total number of *Orobanche* shoots indicate that glyphosate was the most effective treatment in reducing *Orobanche* growth and development after 40, 60 and 80 DAPE as compared to the control (Fig. 9). Glyphosate applied at 40 DAPE reduced the number of *Orobanche* shoots by 94%, while ammonia reduced *Orobanche* infestation by 54% as compared to the control. Unlike ammonia gas, glyphosate reduced, *Orobanche* shoot number by 97 and 99%, after 60 and 80 DAPE respectively, as compared to the control. In addition, the observed *Orobanche* shoots were stunted and almost dead. The low efficiency of ammonia gas in reducing *Orobanche* infestation could be contributed to the plowing of the field two days after injection of the ammonia gas; this could have resulted in the escape of volatile ammonia gas, and had a little effect on *Orobanche* seeds in the soil (Simpson, 1986). Sequential application of glyphosate showed to be significantly effective in reducing *Orobanche* infestation; it was shown that frequent spray of glyphosate on parsley completely controlled *Orobanche (*Goldwasser *et al.*, 2003). Glyphosate was also found to be effective against *Orobnache crenata* in faba bean and *Orobanche aegptiaca* in tomato and tobacco when used in successive applications (Kasasian, 1973; Jacobsohn and Kelman, 1980).

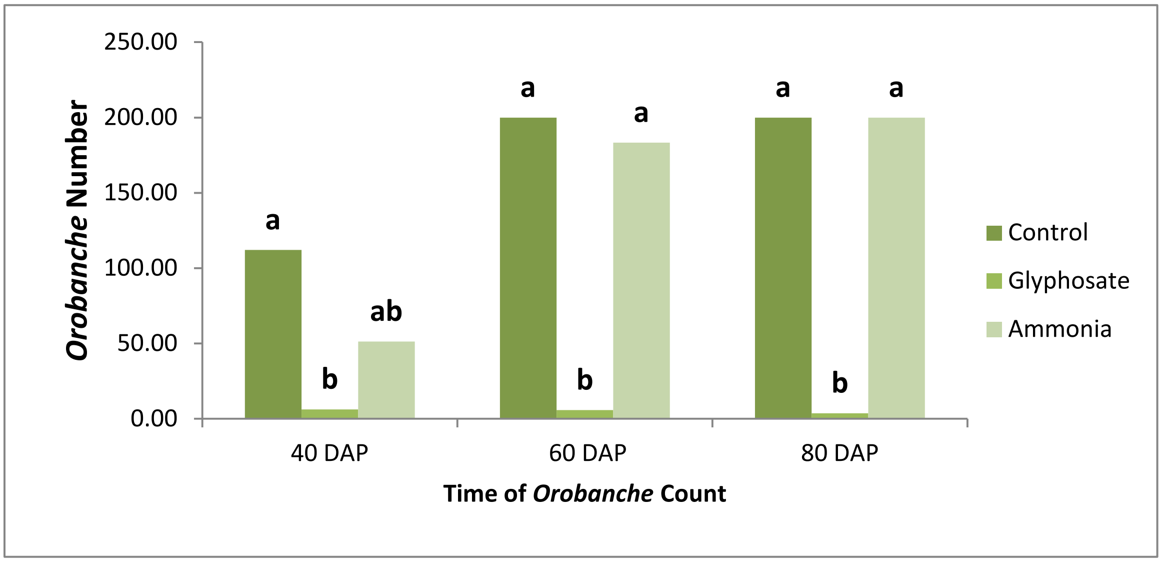

Figure 9. Effects of glyphosate and ammonia on average *Orobanche* shoot number (n = 4). Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.

2. Potato Quantity and Yield

Results in table 6 show that glyphosate at all tested rates had significantly increased potato yield compared to the ammonia treatment and the control. Moreover, the total number of potato tubers was also significantly higher by 63% in glyphosate treated plots than both the ammonia and the control plots. The difference in the number of non-marketable tubers followed the same trends of significance as the potato total yield. Treatment with glyphosate significantly increased the number of non-marketable potato tubers, while no significance change in the number of marketable tubers was recorded. Sub-lethal doses of glyphosate had no negative effect on potato tubers. Glyphosate was previously shown to reside in the potato plant organs and injured potato tubers (Potato Council, 2008). This also demonstrates the results of Worthington (1984) where the collected tubers upon glyphosate treatment showed to rotten, almost dry and full of shallow lesions. The increased number of nonmarketable tubers was previously observed and reported by Lutman and Richardson(1978).

Table 6. Yield response of potato to single or sequential application of sub-lethal doses of glyphosate at 125 g ai/ha and injected ammonia gas at 4, 4.5 or 6 kg/200 m^2.

Treatment	Total Yield (Ton/ha)	Total Tuber Number (per $3m^2$)	Marketable tubers number (per m)	Non-marketable tubers number (per m)
Glyphosate	16.2 a	32.3 a	11.5 a	20.8 a
Ammonia	9.5 b	18.2 b	8.5 a	10.0 b
Control	10.0 b	19.9 b	8.1 a	11.8 b

*Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.

C. Field Experiment Two

1. *Orobanche* Management

Except for single application of glyphosate at 60 and 80 $g.ha^{-1}$ applied at 20 DAPE, all glyphosate treatments significantly reduced *Orobanche* infestation as compared to the control (Table 7). Single application of glyphosate at 100 $g.ha^{-1}$ at 20 DAPE resulted in significant reduction of *Orobanche* by 65% as compared to the control. Sequential application of glyphosate at 60, 80 or 100 $g.ha^{-1}$ at 20, 40 and 60 DAPE were the most effective treatments in reducing (Eradicated) *Orobanche* shoot number after 100 DAPE and short dry weight. These treatments reduced *Orobanche* infestation by 100%, compared to the control (Table 7). These results demonstrate the previous observations that split application of sub lethal doses of glyphosate is recommended for the eradication of *Orobanche*. Zahran *et al.,* (1988) showed that post emergence sprays of glyphosate at 64 $g.ha^{-1}$ effectively controlled *Orobanche crenata* in broad bean fields. Castejon-Munoz *et* *al*., (1990) reported that glyphosate at 20-40 g.ha^{-1} at 12 and 14 days interval eradicated more than 80 % of *Orobanche cemua* in sunflower plots. Halila (1988) observed that 60 ml glyphosate in 500L of water per ha almost completely eliminated *Orobanche crenata* in broad bean and field bean plots. Moreover, Sauerbcjrn *et al.* (1989b) reported that. *Orobanche crenata* and *Orobanche aegyptiaca* were controlled completely due to application of 80 g.ha^{-1}.

Table 7. Average *Orobanche* shoot number and dry weight (n = 4) in response to sub-lethal doses of glyphosate.

Treatment	Rate (g ai/ha)	Application time DAPE	*Orobanche* Shoot number (per m^2) Harvest	Shoot dry weight (g per m^2)
	60	20	31.3 a	20.5 a
	80	20	18.0 a	14.8 a
	100	20	9.0 b	7.8 b
	60	20/40	4.0 b	2.6 bc
	80	20/40	2.3 bc	1.4 bc
Glyphosate	100	20/40	1.0 c	0.7 bc
	60	20/40/60	0.0 c	0.0 c
	80	20/40/60	0.0 c	0.0 c
	100	20/40/60	0.0 c	0.0 c
Control			25.7 a	16.1 a

*Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.

2. Effect on Potato

Similar to the results of the green house experiment, all single and sequential application of glyphosate (60, 80 and 100 g ai/ha) treatments were toxic to potato plant after 60 DAPE in comparison to the control (Table 8). Visual potato injury appeared 10 days after first spraying and included leaf yellowing, plant stunting and compact potato plants, compared to the control. The phytotoxicity visual rating decreased in most glyphosate treatments after 75 DAPE. Single or sequential application of glyphosate at all tested rates had no significant effect on potato height 60 DAPE. However, sequential application of glyphosate at 100g ai/ha was toxic to potato plants and significantly reduced potato shoot height 75 DAPE. It's well known that glyphosate damages the host crop, this is demonstrates by the pervious results by Lolas on tobacco plants (1994). Moreover, foliar injury increased and plant vigor of pepper (*Capsicum annuum*) declined when increased rates of glyphosate where applied, this was also exacerbated by several applications of glyphosate (Gilreath *et al*., 2009)

Table 8.Effect of glyphosate on average potato shoot height and vigor (n = 4)

Treatment	**Rate (g ai/ha)**	**Application time (DAPE)**	**Phytotoxicity visual rating (%)***		**Plant height (cm)****	
			60	**75**	**60**	**75**
Glyphosate	60	20	7.6 bc	8.4 ab	58.3 a	78.4 ab
	80	20	7.1 bc	8.3 ab	59.4 a	78.2 ab
	100	20	6.6 c	7.5 bc	54.3 a	66.2 ab
	60	20/40	7.6 bc	7.6 bc	60.9 a	77.0 ab
	80	20/40	7.6 bc	7.3 bc	61.2 a	68.3 ab
	100	20/40	7.0 bc	6.1 d	56.7 a	64.0 b
	60	20/40/60	8.0 b	7.5 bc	64.5 a	80.7 ab
	80	20/40/60	7.5 bc	7.4 bc	62.3 a	66.5 ab
	100	20/40/60	7.1 bc	6.8 cd	56.1 a	64.0 b
Control			9.0 a	9.0 a	69.2 a	82.2 a

*Scale (0–10), with 0 indicating complete death and 10 no injury
**Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.

3. Effect on Potato Yield

Single or sequential application of glyophosate at 100 g ai/ha significantly reduced potato yield and produced non-marktebale tubers (Table 9). The weight of non-marketable potato increased with the repeated application of glyphosate at 100 g ai/ha, in contrast, the marketable potato weight decreased as compared to the control. Phytotoxicity was mostly reflected in the tuber quality of potato yield grown for fresh market with a high incidence of deformed and small tubers. Single and sequential application of glyphosate at 60 and 80 g ai/ha was selective in potato and had no negative on the tuber quality. These results are similar to the observations by Gilreath *et al.*, (2012) where marketable yield of tomato declined with glyphosate rates at 100 g/ha. It could be suggested by that applying glyphosate at rates between 60 and 80g ai/ha would completely control *Orobanche* infestation and have the lowest effect on potato plant vigor and yield quality. The glyphosate rate of 100 g ai/ha was toxic to the potato plants and decreased the marketable tubers although it showed to significantly reduced *Orobanche* shoot number. These results demonstrate the previous studies by Nadal *et al*., (2008) where a rate of 67g ai/ha of glyphosate applied twice to narbon bean (*Vicianar bonensis* L) completely controlled *Orobanche crenata* infestation and increased seed production of the plant compared to the control.

Table9. Yield response of potato to glyphosate.

	Treatment dose (g ai/ha)	Application time (DAPE)	Total Yield (ton/ha)*	Marketable tubers (ton/ha)	Non-marketable tubers (ton/ha)
Glyphosate	60	20	33.9 a	28.3 a	5.6 a
	80	20	29.9 a	16.6 b	13.3 a
	100	20	25.9 b	22.7 a	3.2 b
	60	20/40	36.0 a	30.9 a	5.1 a
	80	20/40	29.9 a	25.4 a	4.5 a
	100	20/40	25.9 b	20.9 b	5.0 a
	60	20/40/60	36.8 a	28.0 a	8.8 a
	80	20/40/60	30.2 a	23.5 a	6.7 a
	100	20/40/60	20.4 b	14.3 b	6.1 a
Control			26.9 a	20.6 a	6.3 a

*Means followed by the same letter, within each column, do not significantly differ at the 5% level according to the LSD test.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

Three experiments were conducted in the central Beq'aa plain at the Agricultural Research and Educational Center (AREC) of the American University of Beirut (AUB) and AUB green house area in Beirut during the growing seasons between April and August of the years 2009 and 2010 for the purpose of studying the effect of glyphosate and other chemical treatments for the control of *Orobanche ramosa* in potato. The first experiment was conducted in spring 2009 in a naturally infested field with *Orobanche ramosa,* treatments with glyphosate and ammonia gas were used to investigate their effect on *Orobanche ramosa* control and on potato growth and development. The Second experiment was carried out in the green house area at AUB between September 2009 and April 2010. Injection with methyl bromide, foliar application of sub-lethal doses of glyphosate, phosphoric acid and sulfuric acid trails were used to investigate their effect on crop and *Orobanche* growth and development. The third experiment was conducted on the same field in spring of 2010 to further examine *Orobanche ramosa* control with, and potato tolerance to, single or sequential applications of sub-lethal doses of glyphosate.

Results concerning the total *Orobanche* control from the three experiments showed that the sequential application of sub-lethal doses of glyphosate has been found the most effective in controlling *Orobanche* in potato as compared to other chemical treatments as well as the control. Sequential application of glyphosate at 60 and 80 g ai/ha significantly reduced *Orobanche* infestation and had no negative effect on potato yield.

Although, sequential application of sub-lethal doses of glyphosate with selected rates above 125 g ai/ha significantly reduced *Orobanche* infestation, it reduced total potato yield and produced nonmarketable tubers.

Conclusion

It can be concluded from this study that:

1. Methyl bromide and sequential application of sub-lethal doses of glyphosate has been found the most effective in reducing *Orobanche* growth and development in potato.
2. Sequential application of glyphosate at 60 and 80 g ai/ha significantly reduced *Orobanche* infestation and had no negative effect on potato yield.
3. Potato plants and tubers showed to be sensitive to sub-lethal doses of glyphosate above 125 g ai/ha. Above this rate, glyphosate could become toxic to potato plants and affect the quality of the potato tubers.
4. Sulfuric and phosphoric acids were neither effective in controlling *Orobanche* nor toxic to potato plants.

Recommendations

Based on these studies conducted for the control of *Orobanche ramosa* in potato it is recommended that:

1. Sequential applications of sub-lethal doses of glyphosate at 60-80 g ai/ha may be used against *Orobanche ramosa* in the open fields.
2. Further studies should be conducted to determine the effect of sub-lethal doses glyphosate on the potato tuber quality since the high percentage of marketable tubers is an advantage.

3. More future studies on integrated *Orobanche* management plans that include several methods to manage *Orobanche* infestations should be carried out.
4. Further research on the control of *Orobanche* using sub-lethal doses of glyphosate on the number of *Orobanache* haustorial attachments should be carried out as the most important factor for the evaluation of the efficiency of glyphosate on *Orobanche* growth.

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