

MANAGEMENT OF BROOMRAPE (*OROBANCHE CERNUA*) IN  
TOBACCO (*NICOTIANA TABACUM*)

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**MANAGEMENT OF BROOMRAPE (*OROBANCHE CERNUA*) IN  
TOBACCO (*NICOTIANA TABACUM*)**

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STELLINGEN

1. Scientific pest control requires a basic knowledge of ecological principles, biological intricacies of the pest, and natural factors that tend to regulate population dynamics of the pest.
2. The longevity of seeds of *Orobanche* spp. in the soil is much shorter than is often stated in the literature. This may strongly affect views on control strategies.

Puzzilli M., 1983. Tobacco broomrapes and their control and some useful references to other parasite and host species. *Rivista Agricoltura Subtropicale e Tropicale* 77, 209-248.

Krishnamurthy, G.V.G., K. Nagarajan & Ramji Lal, 1985. Studies on longevity and depth of emergence of broomrape in tobacco field. *Indian Journal of Weed Science* 17 (4), 38-41.

3. The phrase "One year seeding is seven years weeding" certainly holds for *Orobanche*.
4. The germination phase is the most critical phase in the life cycle of broomrape; control should focus on this phase.
5. The positive effects of root exudates on the germination of *Orobanche* seeds in the presence of high concentrations of a strigol analogue suggest that a complex of stimulants is required to obtain maximum germination.
6. Broomrapes reduce yields of host plants by affecting partitioning of assimilates and nutrients, not by a direct toxic effect.
7. Integrated control of broomrape at high infestation levels should include growing a trap crop, chemical control by a selective herbicide and control of remaining spikes by hand weeding or plant oils. At low levels of infestation, use of herbicides can be omitted. Late hand weeding remains essential, even at very low levels of infestation.
8. Development of herbicide resistant crop cultivars through genetic engineering will enhance the use of selective herbicides to control parasitic weeds.

9. To achieve multi-dimensional sustainability in Indian agriculture, an integrated management strategy to control pests, diseases and weeds, and organic farming are the best options, since they are ecologically friendly, environmentally safe and economically feasible.
  
10. Mind is like a parachute: it only functions when it opens.

Stellingen behorende bij het proefschrift:

Management of broomrape (*Orobanche cernua*) in tobacco (*Nicotiana tabacum*)

G.N. Dhanapal,  
Wageningen, 16 september 1996

## **ACCOUNT**

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*Affectionately Dedicated  
To My Family*

Mrs. Pushpa pal  
&  
Master Yashpal



## ABSTRACT

Dhanapal, G.N., 1996. Management of broomrape (*Orobanche cernua* Loeffl.) in tobacco (*Nicotiana tabacum* L.). Doctoral thesis, Wageningen Agricultural University, Wageningen, The Netherlands, 183 pp., English and Dutch summaries.

Tobacco is an important commercial crop in India. India is the third largest tobacco producing country in the world. Tobacco is cultivated in an area of 0.428 million ha. Non-Virginia tobaccos such as bidi tobacco constitute about 65% of the total tobacco area in the country.

Broomrape (*Orobanche cernua*) is a debilitating holoparasitic weed in all tobacco growing areas in India, with a devastating effect on the crop. In India, yield loss in tobacco ranges from 30 - 70%; at present hand weeding is the only practice in India applied to control the parasite.

With this background, several field and laboratory experiments were conducted in Karnataka State, Southern India, to study the germination biology and to develop a suitable method to induce the germination of the parasite, and to develop a technology by integrating agronomic and chemical approaches to control the parasite at different phases.

The germination phase of the parasite is a critical period. The seed bank of the parasite can be reduced by stimulating the germination through chemicals, natural host stimulants or both. GR24 (a strigol analogue) at 1.0 and 0.1 ppm, was the standard to assess potential germination. Of the other chemicals, gibberellic acid at 10 and 20 ppm was most effective. The stimulating effects of host plants were significant even when GR24 was applied. Suicidal germination of the parasitic seeds triggered by growing trap crops reduced the weed population and the growth of the host plants was hastened due to green manuring effect of trap crops. Therefore, including a trap crop in the rotation may reduce the problem. Sunhemp (*Crotalaria juncea* L.) and greengram (*Vigna radiata* L.) are promising trap crops in a cropping system containing bidi tobacco in areas where tobacco is grown in a long growing season.

Chemical control by (systemic) herbicides is also an option. Maleic hydrazide (MH) reduced broomrape spikes at 0.25 - 0.75 kg a.i./ha applied at 30 or 40 days after transplanting (DAT) tobacco. Higher tobacco yields were obtained with 0.25 kg a.i./ha MH, which was on par with the hand weeding treatment both in "infested" and "non-infested" tobacco plants. Higher concentrations of MH were toxic to tobacco crop. Glyphosate at 0.50 kg a.i./ha applied at 60 DAT and imazaquin at 0.01 kg a.i./ha applied at 30 DAT reduced the broomrape population by almost 80% and increased tobacco leaf dry weight by more than 40%

compared to the control treatment. Imazapyr and EPTC were less effective.

Swabbing natural plant oils killed the bud and stem parts of the parasite by suffocation. Neem, coconut and sunflower oils showed quick knock-down effects in killing the bud part, whereas neem oil did not kill the stem part of the parasite. Niger, castor and mustard oils appeared to be (somewhat) less effective.

In general, there is a negative linear relation between broomrape infestation and tobacco yield, with a very large (negative) regression coefficient.

No single method is effective in controlling the parasite. The seed bank of the parasite should be minimized in a phased manner by integrating cultural and chemical methods of control. Therefore, an integrated management strategy is the best perspective to control broomrapes in a crop wherever it is problematic.

**Key words:** bidi tobacco, broomrape, chemical control, *Crotalaria juncea*, gibberellic acid, germination stimulants, GR24, herbicides, integrated weed control, natural plant oils, *Orobancha cernua*, parasitic weed, suicidal germination, trap crop, *Vigna radiata*.

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## CONTENTS

1 GENERAL INTRODUCTION .....	2
1.1 The tobacco crop .....	3
1.1.1 Tobacco growing in India with special reference to bidi tobacco	4
1.1.2 Constraints in tobacco production in India .....	6
1.2 Broomrape weed .....	9
1.2.1 Biology of <i>Orobanche cernua</i> Loeffl. ....	10
1.2.2 Ecological distribution of <i>Orobanche cernua</i> Loeffl. ....	12
1.2.3 Crops affected by <i>Orobanche cernua</i> and crop losses .....	14
1.2.4 Yield reduction in tobacco in India .....	15
1.2.5 Losses in quality of tobacco in India .....	16
1.3 Control of <i>Orobanche cernua</i> Loeffl. ....	16
1.3.1 Constraints to control .....	16
1.3.2 The present practice and potential new approaches of broom- rape control .....	17
1.3.2.1 Physical methods .....	18
1.3.2.2 Cultural methods .....	18
1.3.2.3 Cropping systems .....	20
1.3.2.4 Chemical control .....	21
1.3.2.5 Biological methods .....	22
1.3.3 Management of broomrape in India .....	23
1.4 Objectives of the research .....	23
1.5 Outline of the thesis .....	24
2 MANAGEMENT OF BROOMRAPE ( <i>Orobanche</i> spp.) - A REVIEW .....	31
2.1 Introduction .....	32
2.2 Development and ecology of <i>Orobanche</i> spp. and their relevance to <i>Orobanche</i> management .....	32
2.3 Aims and structure of the review .....	36
2.4 Tools to control <i>Orobanche</i> .....	37
2.5 Control during seed phase .....	37
2.5.1 Physical methods .....	37
2.5.2 Chemical control .....	41
2.6 Control during germination phase .....	41
2.6.1 Physical methods .....	41
2.6.2 Chemical methods .....	42
2.6.3 Biological methods .....	45
2.6.4 Other cultural measures .....	48

2.7 Control during the parasitic (reproduction) phase	50
2.7.1 Physical methods	50
2.7.2 Chemical methods	51
2.7.3 Biological methods	54
2.7.4 Other cultural methods	55
2.8 Integrated control	55
2.9 Conclusion	56
3 FIELD OBSERVATIONS ON INTERACTIONS BETWEEN <i>OROBANCHE</i> <i>CERNUA</i> LOEFL. AND BIDI TOBACCO IN NIPANI, INDIA	74
3.1 Introduction	74
3.2 Materials and Methods	75
3.3 Results	76
3.3.1 Rate of development of <i>Orobanche cernua</i>	76
3.3.2 Growth of <i>Orobanche cernua</i> and tobacco	76
3.4 Discussion	84
4 BROOMRAPE CONTROL BEFORE ATTACHMENT TO HOST THROUGH CHEMICALLY OR BIOLOGICALLY MANIPULATING BROOMRAPE GERMINATION	89
4.1 Introduction	89
4.2 Materials and Methods	91
4.3 Results	95
4.4 Discussion	101
5 STUDIES ON THE POST-EMERGENCE CONTROL OF BROOMRAPE IN TOBACCO WITH HERBICIDES	108
5.1 Introduction	108
5.2 Materials and Methods	109
5.3 Results	114
5.4 Discussion	123
6 REDUCTION OF INFESTATION OF BROOMRAPE ON TOBACCO BY METABOLIC INHIBITION USING MALEIC HYDRAZIDE	129
6.1 Introduction	129
6.2 Materials and Methods	130
6.3 Results	132
6.4 Discussion	136

<b>7 STUDIES ON THE POST-EMERGENCE CONTROL OF BROOMRAPE WITH NATURAL PLANT OILS</b> .....	140
7.1 Introduction .....	140
7.2 Materials and Methods .....	141
7.3 Results .....	143
7.4 Discussion .....	144
<b>8 BROOMRAPE CONTROL IN A CROPPING SYSTEM CONTAINING BIDI TOBACCO</b> .....	148
8.1 Introduction .....	148
8.2 Materials and Methods .....	149
8.3 Results .....	154
8.4 Discussion .....	161
<b>9 GENERAL DISCUSSION</b> .....	167
<b>SUMMARY</b> .....	174
<b>SAMENVATTING</b> .....	179
<b>CURRICULUM VITAE</b> .....	183

# Chapter 1



## Chapter 1

### GENERAL INTRODUCTION

As a foreign exchange earner, tobacco is an important commercial crop of India, playing a major role in the Indian economy. It is providing employment to nearly six million people annually in crop production and manufacture of tobacco products both in urban and rural areas (Anonymous, 1995). Tobacco is a unique crop in that quality is as important as yield. Hence in its cultivation, special attention is paid to the production of a good crop with healthy and high quality leaves. Attack by diseases, insects and weed will reduce the leaf yield and leaf quality.

Like any other crop, tobacco is not spared from the ravages of pests. One of these is the flowering holoparasite called broomrape (*Orobanche cernua* Loefl.). It is a serious root parasite on tobacco in India, threatening the livelihood of the tobacco growers with its devastating effect on the crop. The parasite occurred in alarming severity in the year 1945 and a legislation was promulgated by the then Madras State, the present Tamil Nadu. The remedial measures suggested were removal of broomrape shoots before seed set and burying them in pits or burning. The legislation was only a partial success due to many limitations and the parasite continued to pose a serious problem even until today. The parasite appeared in epidemic level in the year 1968 destroying the entire tobacco crop in the East Godavari district of Andhra Pradesh (Krishnamurthy et al., 1994). Presently, yields are being drastically affected in the Nipani area of Karnataka State. Extensive studies on *Orobanche cernua* and its effects on tobacco were performed by Krishnamurthy et al. (see e.g. Krishnamurthy, 1994). However, these concern another cultural system than the bidi tobacco growth in the Nipani area. Since detailed studies on various fundamental and applied aspects of this parasite are lacking, such studies may be of immense value to evolve effective, safe and economical methods of control. Therefore, a study entitled 'Management of Broomrape in Tobacco' was planned and conducted at the Agricultural Research Station, Nipani, India where *Orobanche cernua* is causing problems in the cultural system in bidi tobacco of the Nipani area. It covers various aspects such as the biology of broomrape, techniques on parasitic seed germination, post-emergence control of broomrape with herbicides and natural plant oils, identifying trap crops in a cropping system containing tobacco; these are described in the forthcoming chapters. However, first some general information on tobacco cultivation with special reference to bidi tobacco and constraints in tobacco production in India is described in section 1.1 of this chapter. Also the biology, ecological distribution of

broomrape and yield losses, the present practice in India and the potential new approaches to control the parasite are described in sections 1.2 and 1.3 of this chapter. A detailed review on *Orobanche* and management of broomrape problems is given in Chapter 2.

### 1.1 The tobacco crop

The origin and history of tobacco (*Nicotiana tabacum* L.) suffer generally from ambiguity and contradictions. According to one source, tobacco was in existence in Asia during the 12th century, when it was not known elsewhere. However, it was Christopher Columbus who discovered the narcotic qualities of tobacco accidentally in the course of his American voyage in 1492. He took some tobacco seeds from the Islands of Tobago and introduced them into Europe. The tobacco plant was first introduced into Europe in the year 1560 by a Spanish physician sent to Mexico. At that time, Jean Nicot, the French Ambassador to Portugal found tobacco in Lisbon and introduced it to the French Court. The botanical name of the plant *Nicotiana* and the word nicotine have been derived from his name. Tobacco is said to have been introduced into India in the beginning of 17th century. The habit of smoking spread in several countries during the 17th century.

Tobacco is consumed in one of many forms all over the world; possibly it is the most democratic luxury and as such it is "a rich man's solace and a poor man's comfort". The trends in tobacco consumption in recent years show more preference towards smoking than other forms. It is estimated from an FAO study (Kori et al., 1993) that an annual increase of 2.5% tobacco production will occur in the 21st century in developing and underdeveloped countries including India.

Tobacco, depending on its curing and consumption purpose, is mainly classified into Virginia tobacco (used for cigarette manufacture) and all other, non-Virginia types (comprising bidi tobacco, tobacco for chewing, burley, natu, hookah, cigar, cheroot and snuff; see below). The difference is due to some biological and chemical characteristics of the varieties (e.g. the rather low content of nicotine in the Virginia tobacco) as well as to the curing procedures.

Curing in tobacco refers to drying of harvested tobacco leaves in order to produce dried leaf of required physical and chemical properties. **Flue-curing** refers to curing of tobacco leaves in tobacco barns under controlled conditions of temperature and relative humidity. It is done to produce Virginia tobacco. **Air-curing** refers to more variable production systems, e.g. cutting the stalk and then curing under atmospheric conditions in air-curing sheds. In India, however, tobacco is harvested by stalk cutting and cured in the sun.

Tobacco is grown in India for nine different purposes or products. They are:  
Bidi: a small quantity of rough tobacco rolled in a leaf (usually the leaves of ebony

tree; *Diospyros melanoxylon* Roxb.), cylindrical in shape but flattened at one end for smoking.

Burley: a thin-bodied, air-cured tobacco varying in colour from buff to chocolate, high in content of alkaloids and nitrogenous constituents grown mainly in Kentucky and neighbouring states and used in cigarettes and to a lesser extent in plugs and smoking mixtures.

Cigar: a compact roll of tobacco leaves, often tapered at the ends, used for smoking.

Cigarette: a small quantity of fine tobacco rolled in paper (usually rice paper), cylindrical in shape for smoking.

Cheroot: a kind of cigar with blunt ends, and thicker at one end than at the other.

Chewing tobacco: tobacco usually in the form of a plug, that contains a large percentage of flavouring material.

Hookah: a pipe for smoking that has a long flexible tube whereby the smoke is cooled by passing through water.

Natu: natu tobacco is a sun-cured tobacco used in the manufacture of cheap cigarettes as well as pipe mixtures.

Snuff: a preparation of powdered tobacco taken up into the nose by sniffing or applied to the gums with a snuff stick.

#### 1.1.1 Tobacco growing in India with special reference to bidi tobacco

Tobacco belongs to the family of the Solanaceae and is one of the most important commercial crops of India. Out of 60 recognised species only *Nicotiana tabacum* L. and *Nicotiana rustica* L. are cultivated extensively. India grows both species, but the largest area is under *Nicotiana tabacum* which is grown all over the country. Since *Nicotiana rustica* requires cooler climates, its cultivation is confined to the northern and north-eastern parts of India (Punjab, Uttar Pradesh, West Bengal, Bihar and Assam).

India is one of the principal tobacco producing countries in the world, being the third largest after the People's Republic of China and the USA. In India, tobacco is cultivated in an area of 0.428 million ha occupying second place for area and third position for production with 580 million kg in the world during 1992-93 (Anonymous, 1995). Thus, India accounted for about 8% of the world area under tobacco and for about 7% for world tobacco production during 1993. Though tobacco is one of the important commercial crops of India, it is being grown only in 0.23% of the total cropped area in the country. During 1992-93, tobacco products alone contributed an excise revenue of 31.05 billion Indian Rupees to the National Exchequer and 5.08 billion Indian Rupees through foreign exchange.

Non-Virginia tobaccos constitute about 65% of the total area and about 73% of the total tobacco production in the country. The lion's share of these uses is taken

by bidi tobacco with 29% of the area and 30% of the production (Anonymous, 1995). Figure 1 depicts the growing areas of both Virginia and non-Virginia tobaccos in India. The Charotar area of Gujarat comprising the Kaira and Baroda districts and the Nipani area of the Belgaum district in Karnataka and the Kollapur and Sangli districts of Maharashtra are the main zones of cultivation of bidi tobacco in the country. The Kurnool area of Andhra Pradesh is a new developing bidi tobacco area. To a small extent bidi tobacco is also grown in Rajasthan and Madhya Pradesh.

Karnataka state is the third largest tobacco growing state in the country; it is producing 20 - 25 million kg of bidi tobacco in an area of 30,000 hectares. Almost 90% of the total area in the Nipani area of the Belgaum district was covered with the crop during 1992-93 (Anonymous, 1995). The important varieties grown in the Nipani area of Karnataka are 'S-20', 'NPN-190', 'Anand-119' and 'PL-5'; the quality of the leaves is considered to be superior as compared to the leaves produced from other parts in the country.

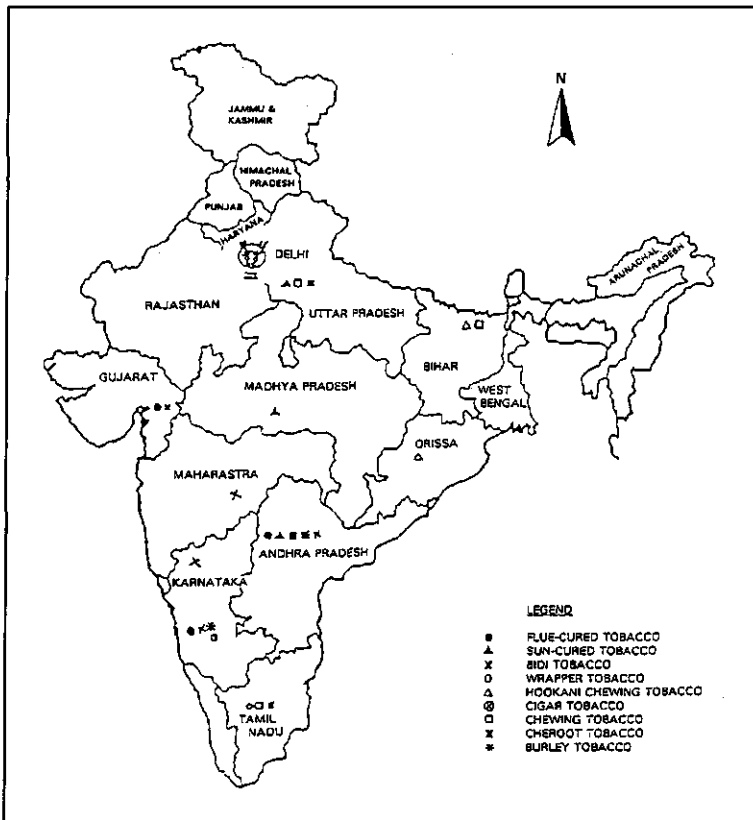


Fig. 1. Tobacco map of India.

### 1.1.2 Constraints in tobacco production in India

The average yield of tobacco per hectare in India is about 1060 kg/ha which is lower than yields in developed countries like Japan (2406 kg/ha), Korea (2326 kg/ha) or USA (2317 kg/ha) (Hosmani, 1992). Experience of tobacco production in India shows that soil and climate are very important factors determining the suitability of a region for commercial tobacco crop cultivation.

#### Soil types

The soil characteristics, particularly the texture of the surface and sub-soils influence the type and quality of tobacco leaves. In India, Virginia tobacco is cultivated on black clay soils, sandy loams and red soils. Black soils offer good condition for tobacco growth, except for these soils in the coastal plains of Andhra Pradesh. Sixty percent of the tobacco area is grown on the black clay soil of the coastal plains of Andhra Pradesh. These soils are not suitable for the cultivation of Virginia tobacco due to their low permeability, poor drainage and high pH values ranging from 7.4 to 8.7. Hence, it is possible only to cultivate tobacco as a dry winter crop on residual moisture.

In the Charotar zone which comprises the Kaira and Baroda districts, bidi tobacco is grown on sandy to sandy loam soils, whereas in the Nipani area of Karnataka bidi tobacco is grown on heavy soils ranging from silt loams to clays. The water holding capacity of the Nipani soil is higher but the air and water permeabilities are poor, thus tobacco yields are reduced. In Western Karnataka comprising mostly lateritic soils, areas are being developed for commercial cultivation of tobacco, including sandy loams and red soils area because the tobacco produced from these light soils is found to be of superior quality.

In Uttar Pradesh, hookah tobacco is grown mainly on the alluvial soils but these soils are saline and need regular irrigation. The hookah-tobacco soils of Bihar State are alkaline silt loams and are highly calcareous.

Chewing tobacco is produced on the gravelly to sandy loams and coastal sands of Tamil Nadu State. Some of the cigar and cheroot tobacco varieties, grown on red loams of the Madurai district of Tamil Nadu, are being used for chewing tobacco because of their poor burning quality.

Natu tobacco grown on heavy clay soils of the Guntur district of Andhra Pradesh is raised on the residual moisture, as an unirrigated crop and these soils are rich in lime. Therefore, a moderate quantity of manuring for heavy clay soils and a heavy manuring for the light soils under irrigated condition are required.

Cigar tobacco is grown on sandy to loamy well drained, red and brown soils of Tamil Nadu; such soils need frequent irrigation.

The burley tobacco is grown on red and sandy loam soils of Andhra Pradesh, which are poor in organic matter and have a low water holding capacity and

fertility.

Irrigation affects the quality of the tobacco produced. The tobacco trade is apprehensive that indiscriminate use of water -sweet or saline- is likely to impair the quality of tobacco (for example, due to high chloride content in the water of Nagarjuna sagar in Andhra Pradesh, the water is found to be unsuitable for irrigating the tobacco areas).

#### Agro-ecological zoning

Besides soil, the other important physical factor which affects tobacco growth is the climate. Rainfall, temperature, relative humidity, wind and sunlight influence the growth, flowering and metabolism of the tobacco plant. To maintain the turgidity and expansion of the leaf and to meet the transpirational losses of moisture from their large leaf area, tobacco plants need considerable amounts of water. On the other hand, tobacco plants are sensitive to flooding or water logging because of deprivation of soil oxygen. Hence, heavy clay soils are ruled out for tobacco cultivation during the monsoon (rainy period), the crop can only be planted in late spring at the end of the rainy season. It grows under residual moisture and protective irrigation. Light soils can be used to raise the tobacco crop provided the rains are evenly light and well distributed during the growing season. This is the case with the Virginia tobacco in Karnataka where it is grown under rainfed conditions during May-September. A few supplemental irrigations are also given when rains are inadequate. In Andhra Pradesh tobacco is grown on light sandy soils completely under irrigated conditions during the dry months in the winter season.

Tobacco in India is grown between 34° N latitude and 8° S latitude and in each major tobacco growing region the cropping season is chosen to have a suitable temperature range for the tobacco type, for instance, 23 °C for the Virginia tobacco. This period of suitable temperature does not coincide with the rainy season except in the Virginia tobacco crop in Karnataka and the cigar and chewing tobacco crops in Tamil Nadu.

The rainy season in India is of the monsoon type and the rains are heavy and unevenly distributed during a limited period of the year (May-October for the southwest monsoon). The atmospheric temperatures during this period in the different tobacco growing areas in India, except in Hunsur (Karnataka) are beyond the optimum for ideal growth of tobacco plant. Tobacco, particularly Virginia tobacco, growing in India during monsoon is susceptible to diseases like leaf-spot and thereby yields are reduced. If the crop is grown in the rainless winter period of October to February, when the temperatures are suitable as in Andhra Pradesh, the soil moisture reserve in the Indian heavy black soils is insufficient to meet the crop demands for full growth and expansion of the leaf. Supplemental irrigations to supply the required moisture to the soil can neither be heavy nor frequent on these

soils. Consequently the yields of Virginia tobacco on black soils are severely reduced. Allowing the tobacco crop to mature in the winter season has its own drawbacks in that the leaf thickens and becomes rugged, which is desirable for bidi tobacco but undesirable for Virginia tobacco. Maturation and curability of the leaf are also impaired because of the decrease in relative humidity during the winter season.

#### Climatic constraints

In India, the cultivation of tobacco crop is a gamble with the climate particularly in respect of water and temperature requirements. Mid-season corrections of any deficiency in climate are virtually impossible in the heavy black clay soils of Andhra Pradesh, where the large quantities of the country's flue-cured tobacco is produced. Prolongation of monsoon well into the tobacco transplanting period may delay the planting and affect the crop adversely. In contrast, a tobacco crop grown in a drought year is thick, dark coloured and has higher nitrogen and nicotine and is inferior in taste. Light showers in the early stages of the crop after transplanting tobacco are a boon, heavy and unexpected rains late in the season at the time of maturity of the crop affect the chemical composition and result in a poor quality of the leaves. In North India, when the mean temperature falls below 5 °C, severe chilling injury to the tobacco crop occurs.

#### Biological constraints

The important diseases of tobacco prevalent in India are:

In the nursery: damping off (*Pythium aphanidermatum*);

In the field: powdery mildew (*Erysiphe cichoracearum*), leaf blight/black shank (*Phytophthora nicotianae*), root-knot nematode (*Meloidogyne javanica*), leaf curl and tobacco mosaic virus caused by viruses, angular leaf spot (*Pseudomonas angulata*), frog eye spot (*Cercospora nicotianae*) and brown spot (*Alternaria alternata*). Root-knot incidence is increasing every year in Gujarat, Karnataka and on the light soils of Andhra Pradesh where the tobacco is raised in nurseries on a commercial scale. The nursery sites of severe infestation should be discarded and new nursery sites should be selected to raise seedlings. Infection at early stages results in heavy loss in yield and quality (Gopalachari, 1984).

Important insects of tobacco prevalent in India are:

In the nursery: caterpillars, stem borers and cutworms;

In the field: ground beetles, leaf-eating caterpillars and capsule borers.

Among insects, tobacco aphids and white flies are also problematic in tobacco production. They serve as vectors in transmitting the viral diseases. Pesticides recommended for tobacco only should be used for the control of pests.

The lower yields of tobacco in India are also due to weeds. In fact, due to their

manifold harmful effects on the growing of the crop, they rank as the prime enemies in crop production. Broomrapes (*Orobanche cernua* Loeffl.) is threatening the tobacco cultivation in Andhra Pradesh and in Karnataka; the losses vary from 20 - 50% depending on the time of infection and the availability of soil moisture (Krishnamurthy et al., 1977). The broomrape and other monocot and dicot weeds are being controlled by weeding methods which are widely used by farmers. Although the methods may be efficient, they are laborious, time-consuming and expensive. These problems may be increased by temporary scarcity of labourers, particularly during peak periods of labour demand, because the weeding coincides with the field operations in other crops.

Lack of awareness among the tobacco growers in India regarding nursery management, pest management, soil and water management and post-harvest technology results in relatively low productivity of all the tobacco crops in India.

#### Socio-economic constraints

The marketing system for Virginia tobacco is done on a scientific basis. On the other hand, the non-Virginia tobacco growers face innumerable difficulties in marketing their produce at remunerative prices mainly due to lack of a marketing infrastructure. Area and production of non-Virginia tobaccos are not regulated as per the demand for domestic consumption neither for export purpose in order to avoid excess production. Moreover, growing tobacco in unsuitable areas and lack of adequate storage facilities result in low quality produce.

Timely supply of quality seeds of tobacco and other inputs such as fertilizers, pesticides, components of tobacco barn construction as well as adequate finances, etc., to the tobacco growers through government/non-government agencies must be ensured in addition to assuring economic prices for the tobacco produced by the tobacco growers.

### 1.2 Broomrape weed

*Orobanche*, commonly known as broomrape, is a flowering plant that parasitises the root of many economically important crop plants. The genus belongs to the family of the Orobanchaceae and it contains about 150 species (Musselman, 1980); most of these are perennials, but the few weedy species all are annuals with a short life cycle (3 - 6 months). They draw nutrients and water through haustoria that penetrate the root tissue, establish connections with the vascular system of the host plants and disrupt the growth and development of host plants to such an extent that yield losses are a multiple of the yield of the weed.

*Orobanche* is a latin name derived from the greek word *Orobos* meaning pea and *anchcin* meaning strangle. The broomrapes are named after the broomrape



*Orobanche rapum-genistae* which parasitises broom (*Cytisus scoparius*). *Orobanche cernua* Loefl. is the most debilitating root parasite on tobacco occurring in almost all tobacco growing areas in India. It is known by different names in different tobacco growing regions, viz. Tokra or Khumbi in North India, Vacumber or Makarva in Gujarat, Bambaku in Maharashtra, Benkigida or Bambaku in Karnataka, Pokayelikalan in Tamil Nadu, Bodu or Malle in Andhra Pradesh and broomrape in English speaking countries.

### 1.2.1 Biology of *Orobanche cernua* Loefl.

The weedy *Orobanche* spp. are annuals that reproduce by means of seeds that are dark brown, oval and very small (0.35 by 0.25 mm), can remain viable in the soil for up to 20 years (Puzzilli, 1983), but according to Krishnamurthy (1994) longevity is limited to 5 years; one plant can produce numerous seeds. The tiny seeds only germinate under the influence of root exudates up to a distance of a few millimetres from the root of the host plant or certain non-host plants (Kadri and Tewfic, 1956).

*Orobanche cernua* parasitises mainly tobacco (*Nicotiana tabacum*), sunflower (*Helianthus annuus*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*) and brinjal (*Solanum melongena*). The *Orobanche cernua* populations attacking sunflower seem to be unable to attack tobacco and vice versa (Musselman, 1994). According to some authors (cf. Ter Borg, 1994), they should be taxonomically distinguished either as separate subspecies or as two species, indicating the sunflower broomrape as *O. cumana* Wallr. A final decision is waiting for further taxonomic studies. Thus far, papers on *Orobanche cernua* may concern both the taxon attacking tobacco and other Solanaceae as well as the relative growing with sunflower only. When citing literature I will stick to the name which was used by the original author. However, it may mean that the information on "*Orobanche cernua*" will not hold always with respect to the tobacco - broomrape system.

*Orobanche cernua* Loefl. as prevalent in India can be described as follows (Krishnamurthy et al., 1994):

Stem: Solitary, 10 - 40 cm long, round, thickened at the base; stems arising from a common base, brownish yellow, covered with small acute scale leaves which are boat shaped with yellow base and dull brown tips.

Inflorescence: Cylindrical fleshy spike bearing many bluish flowers.

Flower: Bluish, long and curved. Flower size 20 × 5 mm.

Bract: Single, ovate, boat shaped 15 × 5 mm size.

Calyx: Two sepals, separate, each sepal is bifurcated either deeply or shallow at its tip, sometimes not bifurcated, 15 × 4 mm size, both sepals equal in length and the tips are bluish.

Corolla: Long, curved and tubular, 20 × 5 mm size, 5 united petals (2 upper big lips + 3 lower small lips), corolla tips are deep-bluish while the base is whitish.

Androecium: Stamen 4, epipetalous attached to corolla at mid height, di-dinamous 2 + 2, filaments pale yellow with stout base, attached at a mid point dorsally to the anthers. Anther dithecous, lobes pointed at the base, pale yellow, placed slightly below the stigma, rarely in level with stigma, anthers extrose, also introse, anther lobes after dehiscence boat shaped.

Gynoecium: Ovary superior, unilocular, bicarpellary, syncarpous, yellow, normal in size with ovules on parietal placentation, numerous ovules, stigma bifid, trifid and tetrafid, fruit is a capsule containing many reticulate brown seeds, seeds are oval in shape (Krishnamurthy et al., 1994).

Upon germination, the embryo cells which are situated near the micropyle expand out of the testa and form a root like structure called germ tube (Kadry and Tewfic, 1956). If it reaches the root of a host plant, the apex penetrates the root tissue and subsequently makes a connection with the vascular tissues in the root and a haustorium is developed on the host plant. It is assumed that the haustorium is a modified root which has evolved during evolution (Atsatt, 1973). Penetration of the host epidermis involves dissolution of the middle lamella, while penetration through other tissues also employs mechanical pressure that pushes portions of cell walls aside; dissolution of cell walls could be traced in the vascular cylinder of the host roots (Joel and Losner-Goshen, 1994).

The vascular tissues which are formed in the host root become connected with a developing bulb shaped organ, the 'tubercle'. After the vascular tissues are formed completely, the tubercle produces numerous root like organs and gives the tubercle a 'crown like' appearance; these organs are called crown roots. The crown roots attack other host roots on which they produce secondary tubercles. After the production of crown roots, a bud is produced on the tubercle which will subsequently form the main axis and the inflorescence. Cross sections of the main axis show the presence of both xylem and phloem and the cross section of the bracts (scale-like leaves) show spongy-like mesophyll and also vascular strands. The stomata are very much reduced in size and have lost their function, excessive water is released through hydathodes.

In India, in the presence of their natural host tobacco, *Orobanche cernua* seeds germinate during the second week after planting tobacco, the germ tubes infect the roots during the third week and the tubercles develop below-ground sprouts until the fifth week. *Orobanche cernua* shoots start emerging above-ground from the sixth week onwards. Flowering is completed by the eighth or ninth week. Stem drying commences by the tenth week and the dehiscence of the capsule is completed during the eleventh and twelfth weeks. Thus, the life cycle of the parasite is completed in about three months after planting tobacco (Krishnamurthy

et al., 1977). New attachments are formed continuously, and fresh spikes emerge until the death of the host plants. The seeds are disseminated by wind, water, animals and men.

### 1.2.2 Ecological distribution of *Orobanche cernua* Loefl.

In Table 1, the global distribution of economically important broomrapes with their major hosts is presented.

Table 1. Economically important broomrapes (*Orobanche* spp.) with their world distribution and major hosts (Foy et al., 1989; Musselman, 1994).

Species	Geographical distribution	Major crop hosts
<i>O. ramosa</i> L.	Central Europe, Middle East, Mediterranean basin, Southern Africa, Chili, Cuba	tomato, tobacco, potato, hemp
<i>O. aegyptiaca</i> Pers.	Central, South-West Asia, Middle East	broad bean, tobacco, melons
<i>O. crenata</i> Forsk.	Mediterranean basin	broad bean, pea, lentils
<i>O. cernua</i> Loefl.	Eastern Europe, Middle East, former USSR, Indian sub-continent	tobacco, tomato, sunflower, bell pepper
<i>O. minor</i> Smith.	Central and Southern Europe, Middle East, USA, New-Zealand	tobacco, clover

*Orobanche cernua* Loefl. is predominantly occurring in and around the Mediterranean basin, Eastern Europe and the Indian sub-continent. Its area is also extended further southwards into Africa, to Niger (Agadez) in the west and Tanzania and Uganda in the east (Fig. 2). Also there are isolated infestations of *Orobanche cernua* in China and in Australia.

*Orobanche cernua* is appearing in all the tobacco growing areas in India (Krishnamurthy et al., 1977). Figures 3 - 5 are maps of the distribution of *Orobanche cernua*. These maps are based on the examination of the herbarium material, literature and field experience. However, they are only approximations of the actual distribution as the recording of local data from the thousands of herbarium specimens is not possible. These maps help us to understand the pattern of distribution of *Orobanche cernua* and indicate the severity of infestation.

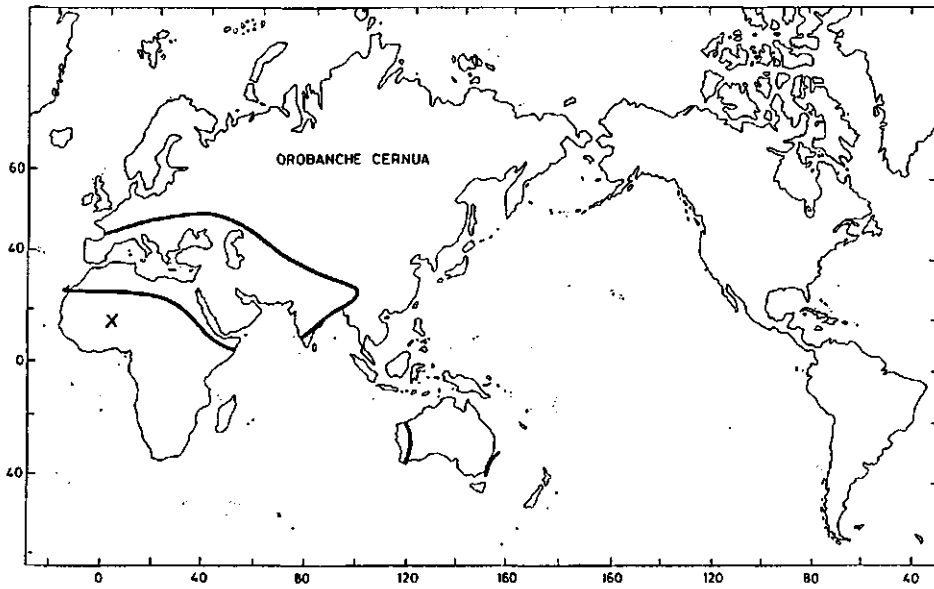


Fig. 2. Distribution of *Orobanche cernua* in the world (from Musselman, 1986).

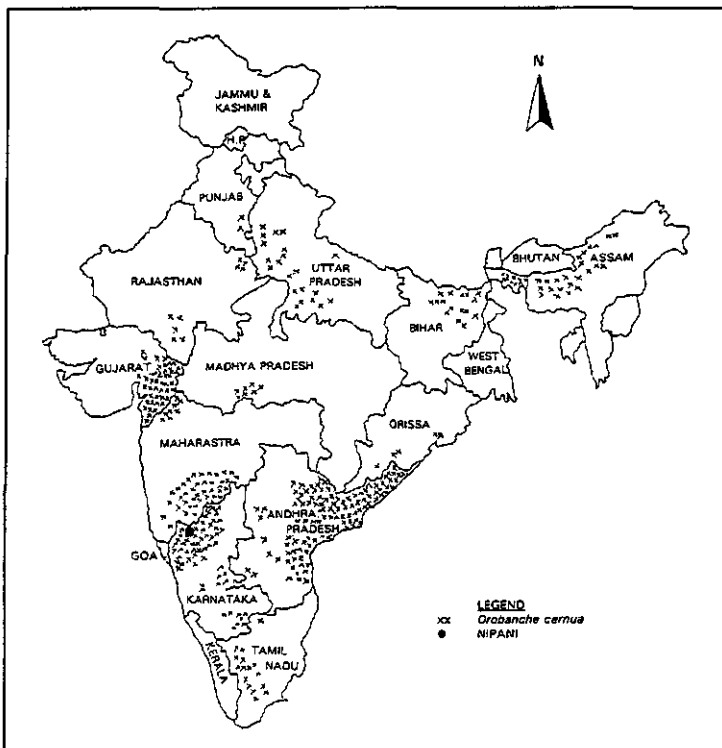


Fig. 3. Distribution of *Orobanche cernua* in India.

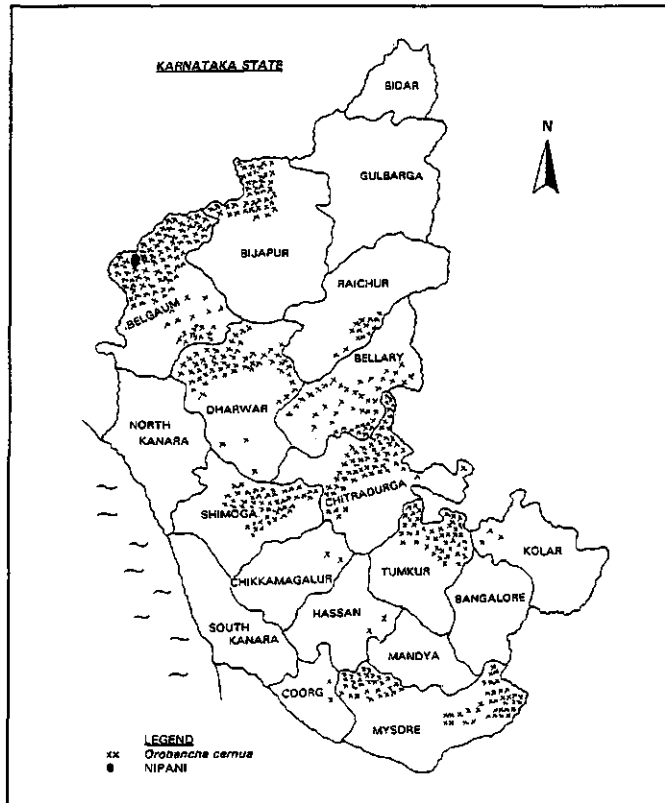


Fig. 4. Distribution of *Orobancha cernua* in Karnataka State.

### 1.2.3 Crops affected by *Orobancha cernua* and crop losses

The range of crops affected by *Orobancha cernua* is quite narrow: it includes the Solanaceous crops such as tobacco, tomato, eggplant, potato; infestation on sunflower will be left out of consideration in this paragraph. Tobacco is seriously affected by *Orobancha cernua* in India and Pakistan and locally in Jordan, Ethiopia, Saudi Arabia and no doubt a number of other countries in West Asia. Tomato is known to be seriously affected by *Orobancha cernua* currently in Israel, Jordan, India and Ethiopia. Eggplant is moderately affected by *Orobancha cernua* in Israel, India and Ethiopia and potato is known to be affected locally in Jordan (Parker, 1994).

In Nepal, *Orobancha cernua* was reported attacking tobacco by Sahu and Sinha (1983).

Yield reduction is not the only form of crop damage caused by broomrape. Another form of economic loss is due to the contribution of broomrape to aberrant

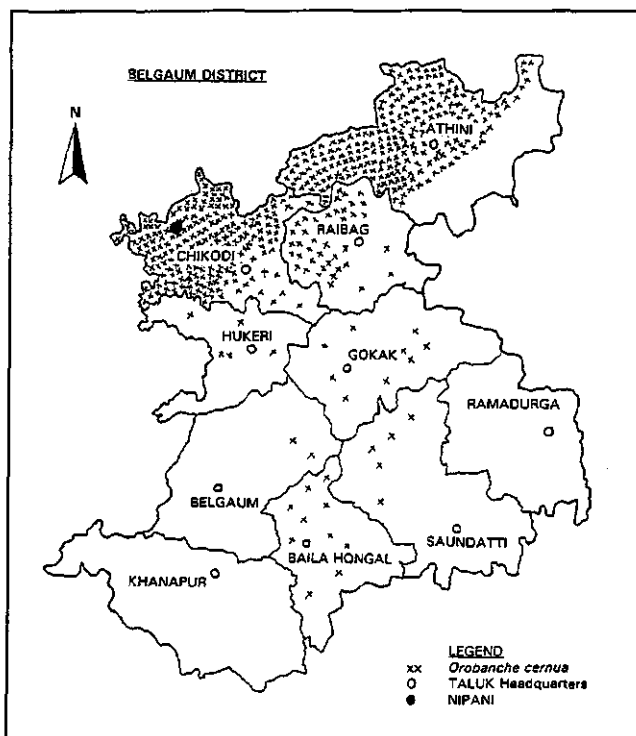


Fig. 5. Distribution of *Orobanche cernua* in Belgaum District.

material in crop products, like broomrape inflorescences in vetch hay and parsley foliage (Foy et al., 1989). Moreover, its presence in the field, if known *a priori*, reduces the number of crop alternatives, and very often second or third-choice crops have to be grown. Also, broomrape may host pests such as tobacco caterpillars (*Spodoptera litura* F.), capsule borers (*Heliothis armigera* Hb.) and green peach aphids (*Myzus persicae* Suiz.), which may damage the crop considerably.

#### 1.2.4 Yield reduction in tobacco in India

In India, about 50% (40,000 ha) of the important tobacco-growing region of Andhra Pradesh is infested. Losses of 25 - 50% are common, and overall yield loss for the region is estimated at 35% (Parker, 1994). The yield losses reported by Mitra (1962) in tobacco elsewhere in the country range from 5 - 10% in Bengal, 15 - 20% in Bombay, 20 - 30% in the Central Provinces, 30 - 70% in Madras and 75% in the islands of the Godavari delta of Andhra Pradesh. Presently infestation level is high in Karnataka, leading to 50 - 60% crop losses, in an area where locally 90% of arable land is cultivated with tobacco.

Tobacco growth, yield and quality of tobacco leaves are all reduced by the *Orobanche cernua* attack, the actual loss being directly related to the incidence and the severity of attack. The infested tobacco plants are stunted in growth and their leaves start drooping as early as 08.00 h in the morning, gradually reaching complete wilting by noon (Krishnamurthy et al., 1977). These authors reported that the crop growth was reduced by 51.0% in the broomrape infested plot and by 19.2% in a plot having both infested and non-infested tobacco plants when compared to a completely non-infested plot. In the infested plot with 100% infestation incidence and a high infestation intensity (average 7.0 broomrape shoots/plant) the losses in yield were 52.4% over a non-infested plot. In a plot with both infested and non-infested broomrapes with 39.3% infestation incidence and relatively low infestation intensity the losses in yields were 24.4% (Krishnamurthy et al., 1977). In another study on broomrape, infestation reduced the plant height by 52.3%, reduced the number of leaves by 34.3%, while 39.5% reduction in dry weight of shoots and 53.7% reduction in dry weight of roots were recorded (Murthy and Nagarajan, 1986).

#### 1.2.5 Losses in quality of tobacco in India

Studies on the effect of *Orobanche cernua* infestation on tobacco showed reduction in dry matter production by about 67% and a reduction in uptake of nutrients by 53%, 78%, 83%, 55% and 66% of N, P, K, Ca and Mg, respectively. The uptake of N, P and K by the broomrape is in the order of 5, 1 and 11 kg/ha. When the total uptake of N, P and K by tobacco plants is considered, it is obvious that although the capacity to take up nutrients is strongly reduced by the infestation only 1/5 to 1/8 of the required uptake of the three major nutrients is removed by broomrape. Broomrape contains about 9% reducing sugars, so the parasite has a large need for the assimilates of the host. This large depletion of sugars reduces the quality of tobacco leaf (Prasad Rao and Murthy, 1976). Physical characters such as shattering index and leaf thickness were reduced considerably in the broomrape affected tobacco leaf.

### 1.3 Control of *Orobanche cernua* Loeffl.

#### 1.3.1 Constraints to control

Broomrape control has as yet only been successful to a limited extent and in some regions the problem even tends to increase. The control of broomrape is beset with numerous difficulties (Foy et al., 1989):

- i. The parasite produces large quantities of tiny seeds.
- ii. The seeds remain viable in the soil for long periods, possibly up to 20 years (Puzzilli, 1983; Linke et al., 1989), although Krishnamurthy (1994) reported that

seed longevity is limited to 5 years only.

iii. In general, parasitic seeds only germinate if a suitable host is present; germination is being triggered by compounds exuded by the host's roots (Koch, 1887) and these exudates are probably active only over a short distance (few millimetres) from the root surface. Hence, most of the seeds in the soil will remain unaffected and viable.

iv. Because broomrape can infect the host from a depth of 30 cm, a great volume of soil must be considered for treatment.

v. The farmer's awareness of the problem arises only when the parasite is emerging; at that time considerable damage to the crop plants has already occurred.

vi. Removal of the parasite by mechanical means is difficult, because it emerges very close to the crop plant. Hand pulling of broomrape is not very effective in heavy infestations, because only emerged broomrapes are removed and shoots of the parasite will continue to emerge over a long period.

It is possible to control the parasite at the seed phase, the germination phase and the parasitic/reproductive phase by practising any one or a combination of the following methods (see also Chapter 2 of this thesis):

1) physical methods which include hand weeding, soil tillage, flaming, flooding/irrigation and solarization;

2) chemical methods, such as the use of soil fumigants, herbicides and germination stimulants;

3) cultural and biological methods which include the use of resistant/tolerant cultivars, crop rotation with trap/catch crops, intercropping and use of insects/fungi, etc.

It is a better option to follow an integrated control strategy to manage *Orobanche* in a crop wherever it is problematic. The various practices presently used in India to control *Orobanche cernua* in tobacco crops and the potential new approaches are described in 1.3.2 in addition to the present practices that are being followed in other countries to control *Orobanche* in various crops.

### 1.3.2 The present practice and potential new approaches of broomrape control

The various practices followed in different countries to control different *Orobanche* spp. are reviewed in Chapter 2. A brief overview of possible methods to control *Orobanche cernua* is given below.

Control measures used in China include hand-pulling, trap-cropping, use of resistant and tolerant varieties, directed spraying with glyphosate and some bio-control by *Fusarium*. In Jordan, hand-pulling is the only control measure in potato fields infested with *Orobanche cernua*. In Israel, control measures include some fumigation and solarization, but the high costs limit the latter technique mainly to



farmers growing organic crops. The practice of combining physical, chemical, cultural and biological methods of control in combating *Orobanche cernua* offers the key to success. Some aspects of control have been investigated under Indian conditions, but only infestation in tobacco is being studied thoroughly. A promising level of control has been achieved locally. However, success obtained in experimental broomrape control in India, by various methods, so far bodes well for the future and needs further information on effects of various methods.

#### 1.3.2.1 Physical methods

Physical methods to control *Orobanche cernua* are helpful in killing or removing the seeds, preventing further development from dormant seed to germinating seeds or killing the broomrape spikes after emergence before flowering. These methods include mechanical and cultural methods.

##### **Hand-weeding**

Hand-weeding is one of the widely recommended methods; it prevents seed production and further build-up of a seed bank. It involves pulling the broomrape shoots out by hand before they flower and burying them in pits or burning them. The weeding operation has to be undertaken once a week. Systematic hand-weeding for 3 - 7 consecutive years completely eliminated the *Orobanche cernua* infestation on tobacco (Pal and Gopalachari, 1957; Krishnamoorti and Krishnan, 1967). This is the most efficient and widely practised method in India for all crops that suffer from this parasite. Yet, it is very expensive, time-consuming and may cause injury to the crop plants.

##### **Implements 'spear' and 'minispear and leaf pusher'**

Recently, Krishnamurthy and Nagarajan (1991) developed the (mini)spear and leaf pusher to control broomrape. The spear cuts the parasite up to 5 cm deep or more beneath the soil surface. This method can be employed any time between the emergence of the parasite and before the onset of flowering. Later, a 'mini-spear and leaf-pusher' were developed to control broomrape on air-cured tobacco crops which have broad leaves hiding the broomrape shoots (Krishnamurthy and Raju, 1994).

#### 1.3.2.2 Cultural methods

This type of control may reduce the damage by increasing the crop tolerance or reducing the seed production of the parasite.

##### **Sowing/planting period**

Although *Orobanche cernua* infestation in several crops is reduced by later

planting, this method appeared not feasible in tobacco due to reduced soil moisture (Krishnamurthy et al., 1976). Research under Karnataka conditions may be useful.

### **Inundation/flooding/irrigation**

Flooding/irrigation/inundation kills the parasitic weed seeds either by preventing the germination and infection or by suicidal germination. In earlier studies, seeds soaked in water for one month lost their viability (Marudarajan, 1950). In a pot study, broomrape (*Orobanche cernua*) infested soil was inundated with water from 1 - 10 weeks, after which the seed was subjected to germination tests. There was a strong decline in germinability as the period of inundation increased (Krishnamurthy et al., 1977). Hence, there is scope to control broomrape by inundation wherever irrigation facilities are available.

### **Soil solarization**

Soil solarization reduces the seed bank of the parasite by increasing the soil temperature. The infestation level could be evaluated during the subsequent cultivation of a crop. To obtain good results, fields should be irrigated before solarization.

In a field study with air-cured tobacco, solarization for 40 days with a 0.05 mm thick, transparent, polyethylene sheet significantly reduced the number of broomrape and increased tobacco yield (Meti, 1993). In a microplot study, *Orobanche cernua* seeds were buried at 7.5 cm depth and subjected to solarization under polyethylene cover for periods up to 28 days, after which the seeds were tested by bio-assay for germinability. The seed germinability which was originally 77.5%, decreased as the period of solarization increased and was completely lost after 7 days of solarization (Krishnamurthy and Raju, 1994). Broomrape control through soil solarization, even though effective, appears not to be feasible under Indian conditions due to the cost of the polyethylene film required and the limited water supply during summer months.

### **Tillage**

The germ tube attachment of the parasite to the host plants usually occurs within 20 cm soil depth. Therefore, some control can be obtained by removing the seeds from the tilth.

In the sandy loams of Gujarat, deep ploughing in summer reduced the broomrape incidence on air-cured tobacco by 30% and significantly increased the tobacco yield over normal ploughing (Khot, 1974). In another study with various depths of planting and timing of the operation, deep ploughing in summer reduced the broomrape infestation by 55.8% - 59.2%, while deep ploughing in the monsoon season reduced it by 26% - 36% as compared with only harrowing

(Krishnamurthy et al., 1987). Deep ploughing in summer also resulted in a significant increase in the tobacco yield (Khot et al., 1987). It is possible in India for the farmers to adopt the deep summer ploughing practice to combat the parasite.

### **Fertilizers**

Broomrapes are more frequently occurring on marginal or poor soils. There are several reports that fertilization, in particular nitrogen fertilization, can reduce *Orobanche* infestation (Kasasian, 1973; Abu-Irmaileh, 1981; Jain and Foy, 1987), but the results of the various investigations are not consistent and often no effect or an adverse effect was observed. Organic manures are also known to reduce the infestation of the parasite.

#### **1.3.2.3 Cropping systems**

##### **Including trap or catch crops in the crop rotation**

The broomrape seed quantity in the soil can be reduced by various cropping systems. In long rotations the seed bank may be reduced by loss of viability with time. Moreover, it may decrease by growing a trap crop which results in suicidal germination; by growing catch crops which are destroyed at harvest before seed production of *Orobanche* the seed bank is also reduced.

A long rotation with tobacco grown once in 3 years preceded by chilli (*Capsicum annum*) reduced the level of broomrape infestation considerably (Marudarajan, 1950). Growing paddy before tobacco also seems to have a controlling effect on broomrape in the succeeding tobacco (Pal and Gopalachari, 1957). When effects of sorghum, maize and paddy were tested, a minimum broomrape infestation was noticed in a sorghum-tobacco sequence followed by a maize-tobacco and paddy-tobacco sequence, while maximum infestation was observed in fallow-tobacco (Krishnamurthy and Umamaheswara Rao, 1976). In a study on the difference between a gingelly-tobacco rotation and a fallow-tobacco rotation, broomrape shoot emergence was less in the former (Ramana Rao et al., 1980).

Crop rotation with non-hosts/trap crops is a feasible method to control the parasite only in a long rainy season, or wherever supplemental irrigation facilities are available. Their planting is most acceptable, if they can be used as fodder or green manure, in addition to reduce the parasite's seed bank in the soil; otherwise, it will be a loss of a growing season. They should be grown at a high seed rate to induce maximum germination of the parasitic seed.

Chilly was found to stimulate broomrape seed germination (Marudarajan, 1950). In another study, crops such as chilly, cotton, deccan hemp (*Hibiscus sabdariffa*),

castor (*Ricinus communis*), sesamum (*Sesamum indicum*), amaranth (*Amaranthus gangeticus*), turmeric (*Curcuma longa*), *Calocasia* spp., sorghum and pearl millet were found to induce the germination of broomrape seed (Rao, 1955). Forty-two crop plants were screened against *Orobancha cernua* in pots for their trap crop effect, and 17 of them (viz. chilli, deccan hemp, wild moong, sorghum, niger (*Guizotia abyssinica*), greengram (*Phaseolus aureus*), bengalgram (*Cicer arietinum*), chicory (*Cichorium intybus*), horsegram (*Dolichos uniflorus*), cowpea (*Vigna cutjang*), redgram (*Cajanus cajan*), blackgram (*Phaseolus mungo*), soybean, cotton, linseed, lucerne and castor) crops were identified as trap crops (Krishnamurthy and Chandwani, 1975; Krishnamurthy et al., 1977). In field studies, Hosmani (1985) found that crops like blackgram (*Cicer arietinum*), greengram (*Vigna radiata*), sesamum (*Sesamum indicum*) and sunhemp (*Crotalaria juncea*) reduced the incidence of broomrape on the succeeding tobacco.

#### **Use of resistant or tolerant varieties**

An attractive approach for tackling the broomrape problem is the selection and use of resistant or tolerant varieties. Unfortunately it is not always possible to find good levels of resistance or tolerance within a crop. Twenty-one tobacco varieties were screened in pots, of which Vattakappal type-G was found to be moderately resistant. Also, 128 varieties of eggplant (*Solanum melongena*) were screened, of which D-12-2-66, DC-4-1-67, E-147, Pusa purple long × Manjri Gota, Pusa purple long × Nurki, Running King and Verma's Giant were found to be highly resistant against *O. cernua* (Dalela and Mathur, 1971). In another study 49 tobacco varieties were screened against broomrape in the field, and none was resistant (Krishnamurthy et al., 1982). This approach is helpful to mitigate the broomrape problem and simultaneously obtain a fairly good yield of the crop without much loss.

#### **1.3.2.4 Chemical control**

Application of herbicides into the soil pre-planting or pre-emergence destroys the seed bank, prevents the germination and disrupts the germinating seeds or advanced stages of the parasite before emergence. Treating the host plant with chemicals may prevent attachment of the parasite. The application of post-emergence selective herbicides before flowering of the parasite may reduce the multiplication of the parasitic weed seeds.

#### **Pre-emergence application**

Many chemicals have been tested for their effects on broomrape. Pesticides such as nemagon, dazomet, methomyl, metham sodium, brestan, thiram, tridemorph, dinocap and carboxin reduced the emergence of the parasite

(Krishnamurthy et al., 1979, 1982). Since soil application is involved, the high cost, pesticide residue problems and environmental problems are discouraging to farmers.

#### **Post-emergence application**

There are several options to control broomrape by using systemic and non-systemic herbicides; in that glyphosate is important. Ascorbic acid (1000 ppm) given as a root dip or foliar spray on the tomato crop completely controlled the emergence of the parasite (Bhargava, 1991). Spraying of 0.1% allyl alcohol on young broomrape shoots led to 66% mortality and soil application of the same chemical at planting points 2 or 4 weeks after planting tobacco suppressed the emergence of broomrape (Pillai and Murty, 1968). Four chemicals, viz. 0.2% allyl alcohol, 0.125% DNOC, 0.125% IC-21 and kerosine led to 43.9%, 20.8%, 30.1% and 88.4% mortality of the parasite in the post-emergence phase, respectively (Krishnamurthy et al., 1976). Fluchloralin, alachlor and pebulate (2 kg/ha) reduced the incidence of broomrape on air-cured bidi tobacco (Palled, 1979).

Glyphosate is a systemic herbicide found effective in controlling broomrape either before or after emergence of the parasitic shoots. It is sprayed on the host plant and kills the parasite by a translocation mechanism. However, its phytotoxicity is a limiting factor. So far, its effects were not tested in India.

#### **Mineral and plant oils**

Swabbing kerosine and diesel oil on young broomrape shoots attacking on opium poppy (*Papaver somniferum*) crop led to 37.5% and 61.0% mortality of the parasite (Ramanathan, 1985). Plant oils from castor, coconut, cotton seed, gingelly, groundnut, linseed, mustard, neem, palm, sunflower, safflower, eucalyptus, pongamia, rice bean, soybean, and tobacco seed and mineral oils such as kerosine, thinner and diesel killed the parasite in the post-emergence phase when 1 - 2 drops were applied directly on the young non-flowering broomrape shoots in a tobacco crop (Krishnamurthy and Nagarajan, 1991; Krishnamurthy and Chari, 1991). Mineral and plant oils have a good knock-down effect on the parasite, but their cost is prohibitive for most farmers.

#### **1.3.2.5 Biological methods**

In general, biological control is an effective method since it is relatively cheap, specific to the target organism and not harmful to the environment.

Extensive surveys to find natural enemies of broomrape were carried out, and many potential organisms have been described. Since some of these are well-known crop pests, they have no specific value as a biological control agent (Samuel, 1940; Narasimhan and Thirumalachar, 1954; Chari and Patel, 1972;

Krishnamurthy and Sitaramaiah, 1974; Manjunath and Sudha Nagarkatti, 1977). So far, no efficient biological agent has been identified for broomrape control in India.

### 1.3.3 Management of broomrape in India

The results of the investigations on the various facets of broomrape control clearly indicate different degrees of success in combating the parasite. This offers scope for the management of the parasite by integrating several methods. Among them, control with the implements 'spear' and 'mini-spear + leaf pusher', inundation, deep summer ploughing, crop rotation with trap (non-host crops) and catch crops (host crops) are the most promising ones under Indian conditions. The following combinations of methods are suggested with four alternatives, dealing with pre-emergence as well as post-emergence control of the parasite (Krishnamurthy, 1994):

1. Inundation + Use of 'spear' /'mini-spear';
2. Deep summer ploughing + Use of 'spear'/'mini-spear';
3. Crop rotation with non-host/trap crops + Use of 'spear'/'mini-spear';
4. Trap cropping with high seed rate + Use of 'spear'/'mini-spear'.

In the above combinations, the first practice is aimed at reducing the seed bank (pre-emergence control), while the subsequent one (i.e. 'spear' or 'mini-spear') is aimed at removing the emerged and non-flowering broomrape shoots (post-emergence control) in the still-infested crops so that seed set is prevented, and thus the field will gradually become free of broomrape seed.

In many cases a further combination of methods may prove to be most efficient, especially, the integration of cultural and chemical approaches, even though it is very costly. Therefore, my research focussed on germination stimulants, post-emergence herbicides and the use of trap cropping.

### 1.4 Objectives of the research

The major objectives of the present study are:

- 1) to understand the germination biology of *Orobancha cernua* in relation to the host crop. This part of the study highlights the complexity of the parasite's etiology, hints at management strategies to be adopted in controlling the parasite, and will help to understand and predict the limitations and benefits of the various control measures.
- 2) to study germination requirements of the parasite, in particular the stimulating effects of some chemicals as well as of root exudates of some potential trap crops and their combination.
- 3) to test the potential of post-emergence herbicides (either systemic or non-systemic) as a control measure.

4) to test the potential of post-emergence application of natural plant oils as a measure to inhibit seed formation.

5) to identify trap crops and test their effects on the build-up of a seed bank under field conditions.

In several field studies, detailed measurements of crop- and broomrape-growth parameters (for "infested" and "non-infested" plants) were carried out to quantify the effects of treatments on the crop and the parasite, at the same time allowing to discriminate between the effects of the treatment on the crop and the effects on the broomrape or on the crop.

Ultimately, the overall study aims at developing a technology by integrating the cultural and chemical approaches to manage *Orobanche cernua* effectively in bidi tobacco in India, based on thorough knowledge of biology and life cycle of the parasite.

### 1.5 Outline of the thesis

In Chapter 2, a detailed review on the management of *Orobanche* spp. is presented. The review highlights the possible ways of controlling the broomrape during the seed phase, the germination phase and the parasitic/reproductive phase of the parasite through physical, chemical and biological methods.

Chapter 3 deals with the life cycle of *Orobanche cernua* in bidi tobacco. The relationships between the parasite and host crop over a period of time are described.

In Chapter 4, a series of laboratory experiments are reported both under incubator and glasshouse conditions in order to maximise the germination percentage of *Orobanche cernua* by using germination stimulating compounds / chemicals, by using various plant species and crop cultivars as trap crops or by using combinations of chemicals and crops.

In field experiments, which are reported in Chapter 5, fifteen systemic or non-systemic herbicides were screened in a primary screening trial to test their phytotoxic effects on the parasite and to test their selectivity in bidi tobacco crop. In the secondary screening, only four herbicides selected were used on a large area to test their potential as a control measure to inhibit the seed formation of broomrape. The effects of the herbicides were analysed, both on the tobacco plant infested and non-infested with *Orobanche cernua* and on the parasite. In another field study, maleic hydrazide was used as a metabolic inhibitor at different concentrations and at different timings to optimise the concentration of maleic hydrazide selective to tobacco but at the same time controlling the parasite (Chapter 6).

In Chapter 7, testing the effectiveness of natural plant oils as a post-emergence

application in preventing the parasitic weed seed formation before flowering is described. In a bidi tobacco field, naturally infested with *Orobanche cernua*, different plant oils were applied on the tip of the parasitic shoot as well as on tobacco leaves and their effects on the mortality of broomrape spikes and on the tobacco leaves were recorded.

Chapter 8 deals with the control of *Orobanche cernua* by means of trap and catch crops. The quantity of biomass of each trap crop incorporated in situ was weighed and their effects on the broomrape population in the succeeding tobacco crop was recorded. Later, at different growth stages of tobacco, various biometric parameters were assessed to quantify the effects of trap crops on the parasite as well as on both "infested" and "non-infested" tobacco plants.

In Chapter 9, the results are discussed and integrated into suggestions for integrated control of *Orobanche cernua* in bidi tobacco.

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## Chapter 2

## Chapter 2

### MANAGEMENT OF BROOMRAPE (*Orobanche* spp.) - A REVIEW

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**Keywords:** broomrape, *Orobanche*, parasitic weeds, chemical control, biological control, integrated weed control

#### Summary

Broomrapes (*Orobanche* spp.) are phanerogamic holoparasites that subsist upon the roots of many important crops thus causing considerable yield losses, especially in the drier and warmer areas of Europe, Africa and Asia.

The major principles of reducing the seed bank and controlling the weed in the germination and parasitic/reproductive phases are critically reviewed. Practices to control broomrape include physical methods (weeding, soil tillage, flooding, irrigation, solarization, flaming), chemical methods (soil fumigation, herbicide application, use of germination stimulants) and biological methods (use of resistant or tolerant varieties, cropping systems with trap and catch crops, intercropping, biological control with insects or fungi). Cultural practices, which help to avoid germination, infection or strong reproduction of the weed or improve the crop's tolerance should be optimized.

However, no single cheap method can control the weed, so integrated management practices are required. Integrated control strategies are site- and cropping-system specific but have in common that measures are taken to kill part of the seed bank, induce the conditioned seeds to germinate in the absence of the commercial crop, kill emerging *Orobanche* shoots before seed set during growth of the commercial crop and further reduce or avoid damage to the commercial crop.

## 2.1 Introduction

Broomrapes (*Orobanche* spp., Orobanchaceae) are a group of noxious phanerogamic, obligate parasites that completely lack chlorophyll and hence the ability to synthesize their own assimilates. Broomrapes are annuals reproducing by means of seeds. These are dark brown, oval and very small (about  $0.35 \times 0.25$  mm, approximately 150 - 200 cells, including the endosperm and the seed coat; 1000-seed weight about 20 mg) and can easily be dispersed by man, wind, water or otherwise.

The genus *Orobanche* contains about 150 species. *Orobanche* spp. commonly parasitizes members of the Leguminosae, Solanaceae, Brassicaceae, Compositae and other dicot families, but generally does not occur on monocots. Among the crops that are seriously affected are faba bean, pea, chickpea, broad bean, and lentil (Leguminosae), eggplant, potato, tobacco, tomato (Solanaceae), safflower and sunflower (Compositae) (see Parker 1994 for review). Host-parasite relationships are affected by environmental factors: some *Orobanche* spp. will parasitize certain crops in one growing season but will not parasitize this crop in another season of the same year (Jacobsohn 1981, Jacobsohn et al. 1991). Although crop rotation avoiding parasite-infected crops would be an effective method of control (Garcia-Torres 1994), the host range is so wide and includes so many economically vital crops and weeds that such crop rotations are often not an option. Yield losses range from 5 - 100%, depending on the level of infestation and the infected crop (Saxena et al. 1994).

*Orobanche* is a serious problem in the warm and dry areas of Africa and Asia, but also in temperate and semi-arid regions of the Middle East, Mediterranean countries and Eastern Europe a few *Orobanche* species are very detrimental and therefore economically important. For the distribution of economically important *Orobanche* species see, for example, Linke et al. (1989) or Musselman (1994).

## 2.2 Development and ecology of *Orobanche* spp. and their relevance to *Orobanche* management

The life cycle of *Orobanche* spp. consists of three main phases: the seed phase, the germination phase and the phase during which the species is parasitic and reproduces itself (Fig. 1).

Details on the seed phase are given in Figure 2. Freshly harvested seeds of *Orobanche* remain dormant for some time, depending on temperature. This phase is also called the after-ripening phase. After dormancy has ended, seeds must be conditioned to germinate, i.e. must be exposed to moist conditions at a suitable temperature during a certain period of time. The optimum temperature for

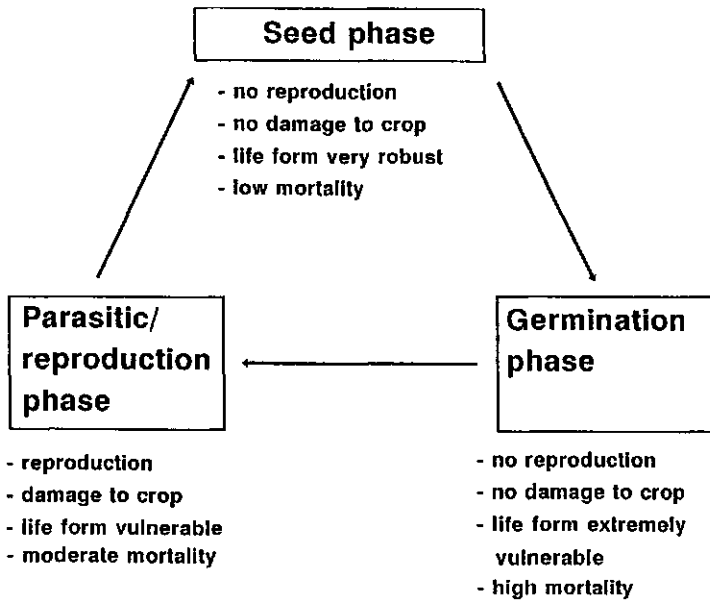


Fig. 1. The three main phases in the life cycle of *Orobanche* spp.

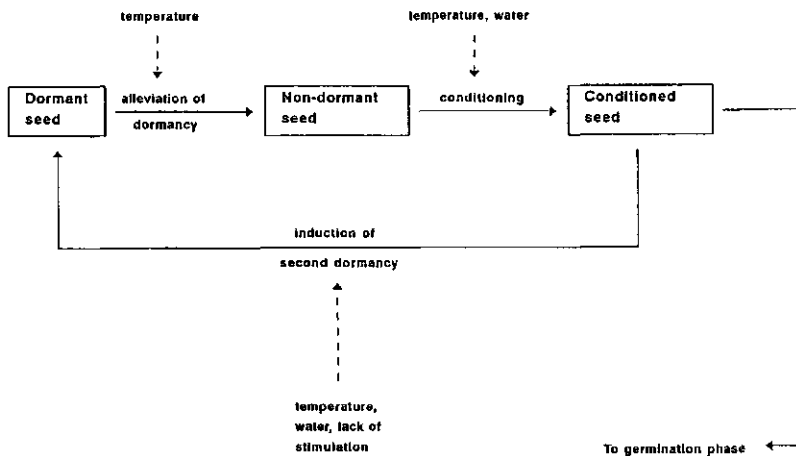


Fig. 2. Steps and processes in the seed phase of the life cycle of *Orobanche* spp.



conditioning is 15 - 20 °C for *Orobancha crenata*; at 20 °C, maximum germination was obtained when the conditioning period lasted 18 days (Van Hezewijk et al. 1993). During wetting either endogenous or exogenous gibberellins are required (Wegmann 1994). When the conditioning period is prolonged or germination is not stimulated, germination is reduced because seeds enter a phase of secondary dormancy (Van Hezewijk et al. 1993). For alleviation of the secondary dormancy, temperature is a crucial factor (Van Hezewijk 1994). Because of the requirements for breaking dormancy and conditioning there is, under field conditions, a large seasonal variation in germinability associated with variation in soil moisture and soil temperature (Van Hezewijk 1994, Van Hezewijk et al. 1994). During the seed phase, *Orobancha* is not able to reproduce or harm the host plant, but it is also difficult to reduce the seed bank with cheap agronomic measures.

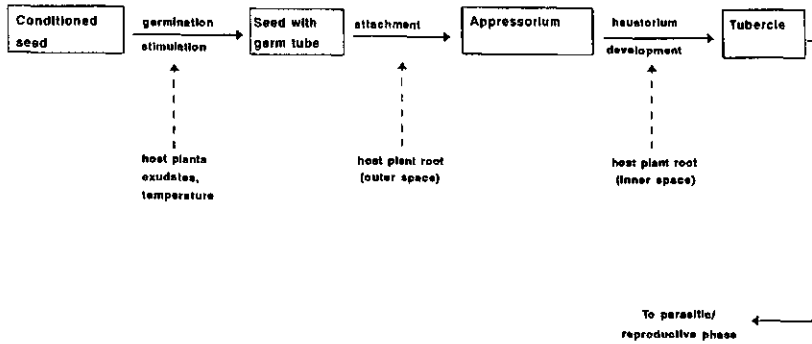


Fig. 3. Steps and processes in the germination phase of the life cycle of *Orobancha* spp.

The important steps and processes in the germination phase are elucidated in Figure 3. After conditioning, germination can only take place in the presence of root exudates containing a germination stimulant (Brown 1946) released by a host plant, and even a few non-hosts (Chabrolin 1935, Brown et al. 1951). Such germination stimulants allow the parasite to recognize the host (xenognosis) and therefore host-derived germination stimulants are also called xenognosins (Boone et al. 1995). The natural germination stimulants are very labile (Boone et al. 1995). The distance between the seed and the exuding root is crucial for successful germination induction. When root exudates are present, the optimum temperature for germination of *Orobancha crenata* is 10 - 20 °C or 15 - 20 °C (Van Hezewijk et al. 1991a). Kasasian (1973a) observed optimal temperatures for *Orobancha aegyptiaca* and *O. crenata* between 18 and 23 °C. Sauerborn (1989) used lentil as

a host plant for *Orobanche aegyptiaca* and *O. crenata*, and sunflower for *O. cumana* and tested the effect of alternating temperatures on germination. He found that the optimal temperature for germination was relatively low (day/night temperature 15/5 °C). Late germination could be observed even at 5/5 °C. High temperatures, 30/20 °C, delayed or inhibited germination, especially in *O. crenata*.

Upon germination a hyaline, root-like structure called the "germ tube" is produced. When the germ tube reaches the host root, it will form a thickening called "appressorium", with which the germ tube attaches itself to the host root. The maximum length of the germ tube is about 3 - 4 mm, meaning that only germ tubes in the immediate vicinity of a host root can attach. If the germ tube does not find a host root within a few days, it will die. The probability of forming haustoria is affected by root amount and root architecture (Ter Borg and Van Ast 1991). Attachment mostly occurs in the soil layer between 1 and 20 cm deep. Sauerborn (1989) observed that at alternating temperatures, the optimum temperature for attachment (day/night temperature 20/10 °C) was higher than the optimum temperature for germination (15/5 °C) in *Orobanche aegyptiaca*, *O. crenata* and *O. cumana*. There was some host plant attachment at temperatures as low as 5/5 °C by *Orobanche crenata* and *O. cumana*. High temperatures (30/24 °C) delayed or inhibited attachment, especially in *O. crenata*.

After attachment a haustorium is produced. A haustorium is an organ that penetrates the host and connects the vascular system of the host with the appressorium. The appressorium develops into a tubercle of 0.5 - 2.5 cm thick which starts to withdraw water, nutrients and organic compounds from the host plant. Attachment and subsequent development of the haustorium might also be triggered by compounds from the host. For *Striga* it has been shown that there is an effect of xenogonins on haustorium induction (Riipel and Timko 1995). For *Orobanche* spp., however, this is still a matter of speculation. Induction of haustorium development can also be triggered or enhanced by thigmomorphogenetic effects.

Germination and attachment are critical events in the life cycle of the weed (Musselman and Press 1995). Because of the vulnerability of the weed during this phase, control measures that affect the weed during these steps are most likely to be successful.

Figure 4 shows the different steps and processes in the phase during which the weed is parasitic and develops its reproductive structures. After crown roots are formed on the tubercle, a bud develops which subsequently forms an emerging reproductive shoot. Before emergence, however, *Orobanche* accumulates reserves so that growth and development after emergence can be very fast. This means that control after emergence will often be too late to prevent considerable damage and

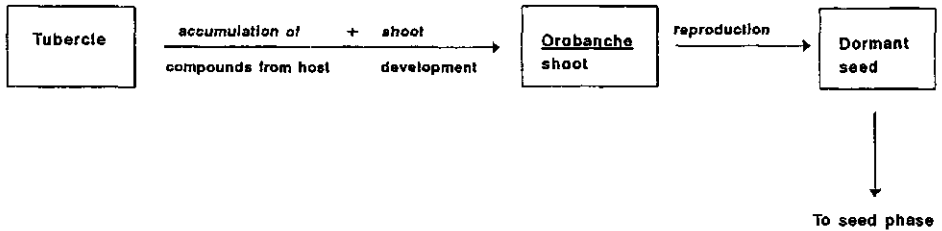


Fig. 4. Steps and processes in the parasitic/reproductive phase of the life cycle of *Orobanche* spp.

is mainly useful to prevent seed formation. After shoot formation the weed will flower and form seeds, which will be dormant immediately after shedding.

Prevention of production of seeds (reproduction) is a crucial aspect of broomrape control. Each broomrape plant is capable of producing numerous seeds, depending on rainfall and soil temperature (Lopez-Granados and Garcia-Torres 1993a). For example, each single *Orobanche crenata* plant can produce up to 500,000 seeds (Cubero and Moreno 1979). Smaller species like *O. ramosa* still produce 5,000 - 20,000 seeds. Seeds of broomrape remain viable in the soil for long periods, possibly up to 20 years (Puzzilli 1983). For *O. crenata* a seed bank of 4 million seeds per m<sup>2</sup> has been reported (Lopez-Granados and Garcia-Torres 1993b).

Due to this enormous seed production and long periods of viability of the seeds in the soil, crop rotation is not an efficient tool for control. One should aim at reducing the parasitic weed population effectively before it becomes unmanageable in the newly infested fields by preventing the formation of new seeds.

### 2.3 Aims and structure of the review

In this paper we discuss the possibilities of preventing the build-up of a seed bank in the soil or reducing the seed bank by physical, chemical and biological methods and other cultural practices. Control of damage to the host plant will not be discussed thoroughly.

The structure will be based on the brief overview of the ecology of the weed given above and on a short overview of the different tools. Different control measures will be described, which act during the different development phases and seize upon different steps or processes indicated in Figures 2 - 4. These control measures will be summarized in Tables 1 - 3 (one table for each main phase in the life cycle).

Based on this information on the effects of single control measures and on the scarce literature on approaches of integrated control, we will discuss strategies for successful integrated control.

In some cases, we will also refer to *Striga* literature.

## 2.4 Tools to control *Orobanche*

**Physical methods** may be helpful in all phases of the life cycle, either by removing or killing seeds, preventing development from dormant seed to germinating seed or killing *Orobanche* shoots after emergence but before reproduction.

**Chemical methods** may involve pre-plant treatment of the soil with chemicals capable of destroying the seed bank, preventing germination or harming the germinated seeds or the later stages of growth of the parasite before emergence. Indirect control of broomrape may be achieved by treatment of the parasite through the host plant just prior to or after attachment to the host roots. Chemical methods should also include control of emerged shoots to prevent reproduction.

**Biological control**, generally effective for a long period, relatively cheap and without harm to the environment, can be very specific to the target organisms and can therefore control the weed without any harm to the crop plants. Methods with potential are still under investigation.

There are also **cultural practices** for controlling the parasite other than the physical, chemical and biological methods mentioned. These practices may reduce the number of *Orobanche* plants and thus reduce damage *and* seed production or may increase the tolerance of the crop.

## 2.5 Control during seed phase (Table 1)

Due to the high numbers of seeds present in the soil, the long viability of these seeds and the limited effects of methods to prevent the production of new seeds, any means to reduce the number of viable seeds present in the soil should be used. Also proper measures to prevent transfer of seeds from one field to another are vital.

### 2.5.1 Physical methods

**Soil tillage:** Most attachments of the germ tube with a host occur at a soil depth of 1 - 20 cm. The parasite can therefore be controlled by removing the seed from the tilth. Labrada and Perez (1988) suggested that in areas with high levels of infestation of *Orobanche ramosa*, deep cultivation with inversion of the soil should

Table 1. Possible control methods during the seed phase of the life cycle of *Orobanche*.

Effect	Methods	
	Physical	Chemical
Removal of seeds from the tilth	Deep ploughing, Zero tillage	
Killing of seeds	Flooding, Soil solarization, Flaming	Fumigants, Drip chemigation
Maintenance of dormancy	Flooding, Irrigation	
Prevention of conditioning	Flooding, Irrigation	
Induction of second dormancy	Flooding, Irrigation	

be practised. Through deep ploughing, followed by minimum tillage *Orobanche* seeds can be buried in the deeper soil layers below the layer where attachments occur. Khot et al. (1987) reported that deep ploughing in summer and monsoon decreased *Orobanche cernua* infestation by 60% and 35%, respectively. Deep ploughing was also reported to be useful for reducing infestation of *Orobanche* spp. in New Zealand (Frater 1975) and elsewhere in India (Krishnamurthy et al. 1987). Trench ploughing to depths of 45 - 50 cm in autumn has reduced *Orobanche* infestation of tobacco in Bulgaria (Alexiev 1967). Deep ploughing to 60 - 65 cm reduced *Orobanche* infestation in tobacco and increased plant height, leaf number and leaf yield (Okazova et al. 1980).

Zero tillage has also been suggested as a control measure (Van Hezewijk 1994). Zero tillage can keep the seeds *above* the layer where attachments occur. However, no effect of zero tillage on the infestation of faba bean by *Orobanche crenata* could be detected by Kukula and Masri (1984) or Nassib et al. (1984).

**Flooding or irrigation:** *Orobanche aegyptiaca* infestation on faba bean in Egypt was found to be reduced in crop rotations including a crop like rice that requires flooding (Sauerborn and Saxena 1986). Cubero (1983) also reported that rotations that include a crop which requires flooding sometimes reduces *Orobanche crenata* infestation. Flooding (inundation) can control the problem of broomrape by killing

the seeds. Zahran (1978) claimed that a two-week flooding prior to sowing gave effective control. Mohamed-Ahmed and Drennan (1994), however, showed that more than six weeks of waterlogging were necessary to obtain proper control of *Orobanche ramosa* emergence. With shorter treatments there was an immediate effect, but after drying and conditioning *Orobanche ramosa* emergence was severe, showing that the seeds were not killed. Short-term flooding or irrigation may induce dormancy, enhance already existing dormancy or prevent conditioning of the seed, thus enhancing the chance of seed decay before germination can occur. Proper irrigation schemes can therefore also control *Orobanche* spp. to some extent (see also Ter Borg 1986a).

**Soil solarization:** A technique of controlling *Orobanche aegyptiaca* by solar heating of the soil has shown considerable success (Jacobsohn et al. 1980). Solar heating is achieved by covering fields with sheets of clear plastic during the hot season. Typically, maximum soil temperatures observed in solarized plots were 8 - 12 °C higher than the ones observed in the uncovered soil (Katan 1980). The temperature increase is realized by a decrease in the soil heat loss, which mainly occurs through convection and evapotranspiration. The maximum increase is observed in the surface layer.

At Hyderabad (India), soil cover with 100  $\mu$ m transparent polyethylene for 6 - 8 weeks increased the soil temperature by 6 - 10 °C in the 0 - 20 cm profile (Chauhan et al. 1988). Soil temperatures at 15 cm in six soils of Davis, California, increased by 10 - 12 °C (Stapleton et al. 1985). In studies at Dharwad (India) the maximum soil temperature increased by 10 - 14 °C due to 0.025 - 0.05 mm thick transparent polyethylene, by 5 - 10 °C due to 0.05 - 0.10 mm thick transparent polyethylene and by 2 - 5 °C by black polyethylene (Emani 1991, Harti 1991, Habeeburrahman 1992). Egley (1983) even observed that the maximum temperature at 1.3 cm during the hottest period of the day increased to 65 °C in a soil at Stoneville, USA covered with transparent polyethylene. Other authors indicate temperature increases at 5 cm depth to 44 °C (Braun et al. 1987), or 45 - 48 °C (Garibaldi 1987).

Maximum temperature in the upper layer is already reached within 4 - 5 days, but it takes longer to reach maximum temperatures in lower soil layers (Kaewruang et al. 1989).

Seven days of solarization seemed adequate for good effect under Indian conditions (Krishnamurthy and Raju 1994a). Other reports indicate that a longer period is required for optimal results. Sauerborn and Saxena (1987) reported that the number of emerged shoots of *Orobanche* spp. per unit area, though initially increased by 10 days of solarization, was strongly decreased by 20 days (50% reduction) or 40 days (90% reduction) of solarization. In Dharwad (India)

solarization of 40 days strongly decreased the infection of bidi tobacco by *Orobancha cernua*, particularly with polyethylene film (Meti 1993). However, the incidence of the weed increased after a 10 day-period of solarization, probably due to a stimulative effect of sublethal temperatures (Meti 1993). In a field study with air-cured tobacco, solarization for 40 days with 0.05 mm thick transparent polyethylene sheet significantly reduced the number and the dry weight of broomrape shoots, resulting in 78% control of the parasite and increased tobacco growth and yield (Meti 1993).

Jacobsohn et al. (1980) reported that mulching with polyethylene sheets of 0.03 mm thick increased the soil temperature by 8 - 12 °C to 56 °C in the top 5 cm of the soil and controlled *Orobancha aegyptiaca* in carrot and eggplant fields. Sauerborn et al. (1989a) obtained best control of *Orobancha aegyptiaca* and *Orobancha crenata* with solarization for 30 - 40 days in the hot season. Maximum soil temperatures under the polyethylene of 0.18 mm thick at 5 cm were 48 °C, 55 °C or 57 °C depending on the year. Broomrape dry weights decreased by more than 90% in both faba bean and lentil fields in the year with a maximum temperature of 55 °C.

In Sudan, soil solarization controlled *Orobancha* by 72 - 100% (Braun et al. 1988). In the faba bean and tomato fields of Egypt, soil solarization controlled *Orobancha* for two successive years (Satour et al. 1991). In Egypt, no broad bean plants were parasitized with *Orobancha* in plots where solarization was applied for 10 weeks before sowing (Abdel Rahim et al. 1987).

Yield increases can be considerable. Linke et al. (1991) observed in field experiments that soil solarization for 40 days increased yields of faba bean, lentil and pea by 331, 441 and 92%, respectively, as a result of the control of *Orobancha crenata* and higher soil availability of nitrogen and phosphorus. Yet, in many cases, soil solarization is not economically feasible.

Mulching of the soil with low density polyethylene sheets (<0.1 mm thick) proved more effective than mulching with black plastic in reducing broomrape (*O. ramosa*) parasitism in eggplant fields (Braun et al. 1984). On the other hand, black polyethylene mulches may have some advantages, especially when direct planting in these mulches after perforation is possible (Abu-Irmaileh 1991).

Usually, the effects of solarization are evaluated by measuring the infestation during subsequent cultivation of a crop. However, it is likely that solarization reduces the seed bank. Fields should be irrigated before solarization for optimal result.

**Flaming:** In the USA, viable seeds and (seed-bearing) whole plants of local infestations of *O. ramosa* are destroyed by surface incineration (Eplee et al. 1994a).

## 2.5.2 Chemical Control

**Fumigants or drip chemigation:** Seeds of broomrape are quite resistant to most chemical control measures except soil treatment by fumigation or drip chemigation (i.e. application of chemicals through drip irrigation).

Fumigants such as methyl bromide, ethyl bromide, metham sodium and dazomet directly kill the parasitic weed seeds in the soil. Soil fumigation with methyl bromide (350 - 500 kg/ha) effectively controlled *O. ramosa* prior to planting tomato and *O. minor* and *O. crenata* prior to planting tobacco and broad bean (Zahran 1970). Zahran (1970) reported that metham sodium (VAPAM) at 500 l/ha provided excellent control of *O. minor* in tobacco and *O. crenata* in broad bean. Di-trapex at 750 l/ha or dazomet granules at 400 kg/ha proved to be less effective than methyl bromide or metham sodium. Jacobsohn et al. (1988) reported that application of ethylene bromide alone or in combination with chloropicrin at the rate of 120 - 480 kg/ha gave effective control of *O. crenata* in pea. Telone II (1,3 dichloropropene) is also promising.

Drip chemigation of metham sodium on dry soils was most effective in controlling broomrape in brinjal (Kleifeld et al. 1991).

The efficiency of soil fumigation and drip chemigation may be increased by a combination with soil solarization. These methods, however, are very expensive and might be hazardous to the environment. In several instances, they are (no longer) legally available for agricultural use.

## 2.6 Control during germination phase (Table 2)

### 2.6.1 Physical methods

**Flooding and irrigation:** Flooding mainly acts through killing the seeds (see above), but it might also act through two other mechanisms: it may prevent germination and infection, or may stimulate seeds to germinate in the absence of a crop which can be infected. Proper timing of the flooding in relation to the planting date of the next crop and the germination behaviour of the *Orobanchae* seeds is crucial. When reducing germination, flooding may have a direct effect by inducing secondary dormancy, enhancing already existing dormancy, impeding the production of germination stimulants or diluting the root exudate. It may also have an indirect effect by reducing oxygen supply, increasing ethylene content, and lowering the soil temperature. These direct and indirect effects may also be observed when the fields are not flooded but properly irrigated (see also above and Ter Borg 1986a).

A decline in germinability of *Orobanchae cernua* seeds with 40 - 49% is noticed under laboratory conditions when the seeds were inundated. The loss of



Table 2. Possible control methods during the germination phase of the life cycle of *Orobanche*.

Effect	Methods			
	Physical	Chemical	Biological	Other cultural
Prevention or inhibition of germination	Flooding, Irrigation	Kinetin, Phenolic compounds, Diphenamid, Trifluralin, Gibberellin inhibitors	Resistant cvs, Intercropping?	Delayed or early or deep sowing, Certain N fertilizers, Manuring
Luring with or without hosts		Strigol analogues, Gibberellins, Ethephon, Ethylene, Other compounds	Trap crops, Catch crops, Resistant cvs, Intercropping?	
Reduction of germ-tube growth		Herbicides, Diphenamid, Trifluralin, Fumigants	Intercropping, Antagonists	N fertilization
Killing of structures before attachment		Herbicides, Fumigants	Intercropping	N fertilization
Prevention of attachment			Resistant cvs	
Prevention of haustorium development or tubercle formation		Ascorbic acid	Resistant cvs	N fertilization

germination was rapid during the first four weeks, but was slow afterwards (Krishnamurthy et al. 1977).

### 2.6.2 Chemical methods

**Chemicals inhibiting germination:** Kinetin and some phenolic compounds in the seeds of broomrape inhibit its germination by inducing dormancy (Cézard 1973a). Saghir and Abu-Shakra (1971) found that diphenamid and trifluralin inhibited the germination of broomrape at a concentration of 10 ppm. This could contribute to reducing the seed bank because a delay in germination may reduce the seed bank.

**Chemicals influencing pre-conditioning:** Since gibberellins are required for the conditioning of the seed to germinate, it has been suggested that gibberellin inhibitors, such as uniconazole, can be effective in inhibiting the germination and thus the infection (Joel et al. 1992a). Zaitoun (1986) observed that *Orobancha oxyloba*, *O. ramosa* subsp. *mutellii* and *Orobancha ramosa* seeds remained ungerminated when treated with water, indole acetic acid or kinetin. Twenty - 120 ppm gibberellic acid as a soaking (pre-treatment) solution or in the germination medium stimulated *Orobancha ramosa* and *Orobancha oxyloba* seed germination, but did not stimulate *O. crenata* seed germination.

Jain and Foy (1987) indicated that conditioning of *Orobancha* seeds in ammonium nitrate alone or in a combination of potassium sulphate and the germination stimulant GR24 at the rate of 1 ppm gave higher per cent germination than conditioning in  $\text{NaCl}_2$ ,  $\text{K}_2\text{SO}_4$ ,  $\text{H}_2\text{PO}_4$  or  $\text{KH}_2\text{PO}_4$  solutions.

**Chemicals inducing germination:** Research on the use of synthetic germination stimulants of *Orobancha* seeds known as strigol analogues or GR-compounds was initiated in the mid-1960s and made great progress in the 1970s (Cook et al. 1966, 1972, Coggon et al. 1973, Johnson et al. 1976, Saghir 1979). Various strigol analogues were tested in Petri dishes for their effect on *Orobancha* seed germination. GR7, GR21, GR28, and GR41 are most active at concentrations ranging from 0.1 - 1.0 ppm (Saghir 1979, Saghir et al. 1983, Saghir 1986). These compounds tend to inhibit germination at concentrations higher than their respective optimal concentrations (Saghir 1979, Janudi 1982). Strigol analogues are chemically very different from the host-derived xenogonins. Germination stimulants may act through their effect on ethylene biosynthesis, which in turn is enhancing germination, as shown for *Striga* (Boone et al. 1995).

Matthews et al. (1991) observed that *Orobancha cumana* was stimulated to germinate only by exudates from its host sunflower (*Helianthus annuus* L.), while *Orobancha aegyptiaca* was more sensitive to exudates from flax (*Linum usitatissimum* L.). This specificity was mediated by different germination stimulants rather than by different species-specific germination inhibitors. The two species of *Orobancha* also differed in their sensitivity to chemically defined germination stimulants, and the magnitude of the difference was greater for GR7 than for GR24. Zaitoun (1986) observed that 0.5 - 15 ppm of the strigol analogue GR7 stimulated germination of *Orobancha crenata* and *O. oxyloba* seed which was pre-treated with water, but *Orobancha ramosa* subsp. *mutellii* seed remained ungerminated. Other researchers reported similar effects of germination stimulants: strigol analogues (GR24 and GR28) stimulated the germination of broomrape seeds (Hiron 1973). Jacobsohn et al. (1988) observed over 90% germination of *Orobancha* spp. conditioned in water and then stimulated to germinate by GR24.

Al-Menoufi and Zaitoun (1987) observed as much as 88% germination in the laboratory when *Orobanche aegyptiaca* seeds were treated with the strigol analogue GR7 at 1 ppm.

In pot experiments Saghir (1979, 1986) and Saghir et al. (1983) found that for most GR-stimulants 1 - 3 ppm was sufficient for proper control. The optimum concentration depended on soil pH and stimulant. Under field conditions, application of GR7 at 0.3 kg/ha in acid soils (pH 4.5) and 1.5 kg/ha in alkaline soils (pH 8.0) was sufficient to reduce *Orobanche crenata* infestation in broad beans (ICARDA 1978). Such application, however, is not feasible in practice. Zwanenburg et al. (1986), however, described attempts to synthesize strigol-like molecules which are biologically active and more stable than the GR-stimulants and the efforts of this research group have continued ever since.

Gibberellic acid ( $GA_3$ ) induced or enhanced germination of *Orobanche* seeds (Hiron 1973, Kasasian 1973a, Kumar and Rangaswamy 1977, Al-Menoufi 1986), which results in the absence of hosts to a reduction of the seed bank. Again, this observation suggests a potential management approach, which is, however, not feasible in practice.

Jain (1987) observed 53% germination induced by ethephon when applied to conditioned *O. aegyptiaca* seeds in the laboratory. In the USA seeds of *Striga* spp. are destroyed by application of ethylene with sophisticated equipment to inject the gas into the soil (Eplee 1983). Similar techniques may also be used for *Orobanche*.

Under aseptic conditions, disinfection of *Orobanche* seeds with calcium hypochlorite induced germination in *Orobanche crenata* (Pieterse 1981), in the absence of root exudate. Maleic hydrazide + ADP and lipoic acid induced germination in certain *Orobanche* species.

Research on identification and standardization of new, stable and cheap analogues of strigol is in progress (Zwanenburg et al. 1994).

**Chemicals reducing germ-tube growth, killing structures before attachment, or preventing haustorium development and/or tubercle formation:** Herbicides that are active in the soil or fumigants can control the weed after germination by inhibiting or reducing germ-tube growth or killing the structures of the parasite before attachment. For example diphenamid and trifluralin reduce the growth of the germ tube at a concentration of 10 ppm (Saghir and Abu-Shakra 1971), but crops will also be killed by this concentration. Ascorbic acid at the rate of 1000 ppm given as a root dip or a foliar spray on a tomato crop induced a chemical resistance and completely controlled the emergence of the parasite (Bhargava 1991).

### 2.6.3 Biological methods

**Luring with non-hosts (trap crops) or hosts (catch crops):** Prevention of build-up of the seed quantity in the soil can be through prevention of the formation of seeds or the removal of viable seeds. Including trap crops (non-hosts) and catch crops (hosts) in the rotation may help to reduce the parasitic weed seed quantity in the soil by inducing suicidal germination or destroying the structures before reproduction.

The use of trap crops was suggested a long time ago (see e.g. Brown 1946), but their use has not been very successful in the past (Nash and Wilhelm 1960). However, interest in this option has increased again.

Trap crops (or false-host crops) induce the germination of broomrape but these crops are not parasitized or, when parasitized, the parasite dies in a very early growth stage. It has been suggested to use vetches, pea, *Carum ajowan* or *Sinapis alba* in the case of *Orobancha ramosa* affecting tobacco; sorghum against *O. aegyptiaca*; *Capsicum annum*, sorghum or cowpea against *O. cernua* affecting tobacco; and alfalfa against *Orobancha* spp. affecting tomato or cabbage (Cubero and Moreno 1979). Hosmani (1985) claimed that the most effective trap crops for broomrape are cowpea, chillies and *Phaseolus tribolus*.

Bischof and Koch (1974) stimulated *in vitro* the germination of *O. aegyptiaca* seeds by chillies. Sorghum, moth bean (*Phaseolus aconitifolius*), cowpea and Deccan hemp (*Hibiscus sabdariffa*) as trap crops stimulated the germination of *Orobancha* seeds to the extent of 50 - 70% (Krishnamurthy and Chandwani 1975).

Krishnamurthy et al. (1977) found in pot experiments that sunflower and safflower allowed *Orobancha crenata* to develop to pin-head sized infections on their roots; chicory, niger (*Guizotia abyssinica*), horsegram (*Dolichus uniflorus*), linseed, alfalfa, soybean, and chickpea were found to induce germination of *Orobancha* seed and received infections that died at the filamentous stage. More research on this topic is necessary.

Catch crops are susceptible host crops which not only induce germination of broomrape but are also parasitized. The parasitization can result in full-grown *Orobancha* shoots and therefore the catch crop and its infections should be destroyed before the parasite flowers. Catch crops (planted in a very high density) may be more effective than trap crops (Sauerborn 1991), although this suggestion is not supported by the data of Kleifeld et al. (1992). The "Silica method" in Spain, described by Cubero (1983), with catch crops in monoculture or in mixture followed by crop destruction by ploughing seems effective in reducing the seed bank of *Orobancha*.

Kleifeld et al. (1994) observed that growing flax (*Linum usitatissimum* L.) in two successive winter seasons or one summer cropping with mung bean (*Phaseolus*

*aureus* Roxbg.) reduced the early infestation of *Orobanche aegyptiaca* and significantly increased tomato (*Lycopersicon esculentum* Mill.) vigour and production. Various sources of *Orobanche aegyptiaca* showed different degrees of virulence to flax, from heavy attack and severe damage to sparse attachment with no production of flowering shoots.

Krishnamurthy et al. (1977) also showed that flax root exudate proved to be effective in inducing the germination of *Orobanche crenata* and *O. cernua* under laboratory conditions. Khalaf (1992), however, found that the active stimulant from flax is only present in the first 8 days after sowing, whereas, for instance, *Vicia faba* shows promotive activity in the period from 30 - 60 days after sowing. When using catch crops, it is therefore crucial to consider the duration of the effect of the catch crop and the sensitivity of the host-plant, especially when sown in mixed cropping systems. The same also holds for trap crops.

In both trap and catch crops, seeds germinate without subsequent reproduction. Because only *Orobanche* seeds in the rhizosphere can germinate, many seeds (about 70%) remain unaffected and viable, and still may cause problems in the next crop (Linke et al. 1991, Sauerborn 1991, Linke 1992). The best trap or catch crops are those that have very high rooting densities and produce ample amounts of germination stimulants. Genetic diversity for such characteristics deserves to be investigated. A high seeding rate of the trap or catch crop is necessary.

Trap or catch cropping is not widely practised because of the loss of a growing season or the need for a long rainy season to allow growth of the commercial crop after the trap or catch crop has used part of the available water. Trap or catch cropping is more efficient, if trap or catch crops could be used as fodder or green manure. In some cases crop mixtures can be more efficient as trap or catch crops than monocultures (Bouhatous and Jacquard 1994).

**Use of resistant cultivars:** The most effective prevention of broomrape parasitism would be by growing resistant cultivars. True resistance means that the *Orobanche* is unable to penetrate the host, or its growth is greatly reduced (Ter Borg 1986b). Resistant cultivars have been reported as early as the 1930s (Beilin 1968) and resistance or tolerance has been reported in many crops, such as sunflower, eggplant, broad bean and vetch.

Resistance can be based upon several mechanisms, such as prevention of germination (e.g. by low stimulant production) or luring with prevention of attachment, penetration (e.g. by mechanical barriers) or tubercle formation (e.g. by antibiosis factors). Resistance based on prevention of attachment or the formation of an effective haustorium would be preferable, since growing a resistant crop would then also contribute to the reduction of the seed bank without damaging the

crop. In fact, resistant cultivars are then used as trap crops (Petzoldt et al. 1994).

Russian breeders devoted considerable time to develop varieties of sunflower resistant to *O. cernua* since 1910 (Pustovoit 1966). S-1358, O-7586, H-8280, Kruglik-A-41 are a few of the resistant varieties of sunflower in a sunflower-*O. cernua* system. Gil et al. (1984) identified resistant lines of *Vicia sativa* (common vetch) to *O. crenata*. Breeding efforts showed that one should not be optimistic about developing resistant tobacco varieties (Puzzilli 1983). Krishnamurthy et al. (1982) screened 49 tobacco varieties for resistance against broomrape and found no resistant type. Moreover, resistance may be lost, as occurred in sunflower in Spain and other countries (Garcia-Torres 1994). This problem can be reduced by rotational growing of cultivars with different types of resistance (Petzoldt et al. 1994). The genetics of resistance, however, are often complex (Cubero 1994, Saavedra del Rio et al. 1994).

Ish-Shalom et al. (1994) showed that there is seasonal fluctuation in sunflower's resistance to *Orobanche cumana*, probably caused by the existence of a temperature dependency in the resistance mechanism.

Genetic engineering can help to develop resistant cultivars (Gressel et al. 1994). Genetic engineering permits the transfer of genes for either herbicide resistance or parasite resistance from wherever they occur into the chosen crop, after the genes have been identified and isolated. Gene constructs or engineered crops already available should be made available to the public sector for the transfer of resistance into the varieties used in areas infested with parasitic weeds.

**Intercropping:** Bouhatous and Jacquard (1994) reported effects of intercropping on the host-*Orobanche* relation. Effects were observed on the number of attachments, and on growth and development of the parasite depending on the crop combinations and the growth of the crop partners. Infection capability of *Orobanche*, however, was enhanced, inhibited or provoked according to the nature of associated species. More research is needed in this area.

**Antagonists:** Gold et al. (1979) reported that certain soils had a suppressive effect on the establishment of *Orobanche ramosa* seeds through biological mechanisms. They also showed that *Rhizoctonia solani* was capable of reducing *Orobanche*, whereas the use of fumigation at a low dose in a commercial field resulted in more vigorous growth of *Orobanche* plants, suggesting that an antagonist had been eliminated. Hodosy (1981) found that *Fusarium solani* and *F. oxysporum* could be used as antagonists for *O. ramosa* with great success. The antagonists caused browning of the haustorium or crown rot.

#### 2.6.4 Other cultural measures

**Sowing:** Previously, we described the effects of temperature on the dormancy, conditioning and germination of *Orobanche* seeds. These effects are relevant for the effects of sowing time of the crops on the level of infestation. Van Hezewijk et al. (1987) proved that indeed the effect of late sowing is associated with a decrease in and retardation of *Orobanche crenata* seed germination as a consequence of the decrease in temperature. The optimum temperatures for conditioning and germination of seeds of *Orobanche* spp. are relatively high.

For winter crops, late sowing in combination with the use of early-maturing cultivars can be practical to avoid the worst effects of broomrape infestation. In Syria, Egypt and Spain, delayed sowing decreased the *Orobanche aegyptiaca* infestation in faba bean (Sauerborn and Saxena 1986). Basler (1981) and Salkini and Nygaard (1983) reported that a delay in sowing of lentil (*Lens culinaris*) until November-December instead of normal sowing in October-November leads to a decrease in infestation by *Orobanche crenata*. Garcia-Torres (1994) indicated that in many winter crops, including broad bean, pea, vetch, chickpea and carrot, the infestation with *Orobanche crenata* decreased progressively when sowing was delayed. In India, Krishnamurthy and Umamaheshwara Rao (1976) observed a reduction in the proportion of infected plants when tobacco was planted late as compared to early planting. Okazova (1975) also reported that more tobacco was parasitized by *Orobanche* in early planting than in late planting.

In spring crops, early sowing can reduce *Orobanche* infection based on the same principal. For example, early sowing of sunflower can increase yield (Castejon-Muñoz et al. 1993). In all cases, late sowing is only economical if early cultivars with reasonable yields are available, so that the yield loss by late sowing is lower than the yield loss caused by *Orobanche* infestation when sown early.

In some crops effects of sowing date on *Orobanche* infection can also be explained by the effect of photoperiod on the development of the crop (Mohamed-Ahmed and Drennan 1994).

Deep sowing of the crop may also reduce *Orobanche* infestation as shown by Kott (1969a) in sunflower. Dense sowing may reduce soil temperatures and consequently reduce *Orobanche* infestation. Such an effect of dense crop populations has been reported for *Striga* (Enserink 1995).

**Fertilizers and manures:** Generally, broomrape occurs on poor soils. This observation prompted several workers to investigate the beneficial effects of various fertilizers and manures. Nitrogenous fertilizers in particular were reported to reduce *Orobanche crenata* in a rate dependent way (Kasasian 1973a, Abu-Irmaileh 1981, Jain and Foy 1987, Eplee et al. 1994b). The underlying

mechanism of this effect is unclear. First of all, fertilizers may act by influencing the pH of the soil, thus indirectly affecting the germination of the *Orobanche* seeds. Saghir (1986) and Saghir et al. (1983) indicated by *in vitro* studies that the activity of the germination stimulant GR24 decreased with an increase in pH, but that the activity of GR28 was not affected by pH. Van Hezewijk (1994), however, clearly showed that an indirect effect on germination is unlikely, since conditioning and germination of *Orobanche crenata* are not very sensitive to changes in pH within normal ranges of soil pH. Secondly, the fertilization may affect the host plant in such a way that infestation is reduced, e.g. because roots produce less germination stimulant, or because dry-matter partitioning is altered resulting in less assimilates moved to the roots. Kasasian (1973b) argued that the effect is due to a reduction in the root biomass of the host plant. It is also possible that the osmotic potential of the host is favourably affected by the fertilizer application, resulting in osmotic injury of the germ tube of *Orobanche* seeds, thus preventing attachment (Abu-Irmaileh 1981, 1985). Alteration in chemical composition of the root tissues of the host may also be important. For example, the concentration of germination inhibitors like coumarin and scopoletin might increase, thus preventing germination of *Orobanche ramosa*. Thirdly, fertilization may also directly affect the *Orobanche* germination as was surmised by Linke (1987), Pieterse (1991) and Jain and Foy (1992) or germ tube growth. Fertilization might also affect the germination indirectly, e.g. through its effects on crop leafiness and thus soil temperature and/or soil moisture.

However, not all the forms of nitrogen are equally effective (Abu-Irmaileh 1985). Linke (1987) found that urea applied during conditioning or germination affected germination and germ tube development, whereas potassium nitrate or ammonium sulphate had no effect. Jain and Foy (1992), however, reported significant effects on germination of applications during the conditioning of either ammonium sulphate or urea. Pieterse (1991) observed a reduction in percentage germination of *Orobanche crenata* from 58% to 5% in the presence of 4 mM ammonium sulphate, whereas the effect of urea was inconsistent and no effects were observed of sodium nitrate. Recently, Abu-Irmaileh (1994) showed that germination of *Orobanche ramosa* under laboratory conditions linearly decreased with an increase in concentration of ammonium nitrate.

Ammonium sulphate and urea markedly reduced germ tube length of *Orobanche crenata* (Van Hezewijk et al. 1991b). In Syria, 40 kg/ha of nitrogen as  $\text{NH}_4\text{NO}_3$  lowered the number and the dry weight of *Orobanche crenata* in faba bean (Kukula and Masri 1984). High levels of nitrogen along with phosphorus and potassium drastically reduced the *Orobanche ramosa* infestation and increased the yield of tobacco and tomato (Abu-Irmaileh 1982a,b).

Systematic *in vitro* research by Van Hezewijk (1994) revealed that during the



conditioning phase only high concentrations of ammonium sulphate reduced the germination percentage. Lower concentrations of ammonium sulphate or other forms of nitrogen (nitrate or urea) did not have an effect. During germination, effects of nitrogenous compounds were more pronounced, but - again - ammonium sulphate had the largest effect. Van Hezewijk hypothesized that the negative effect of ammonium sulphate was caused by a poor ability to detoxify ammonium by a low activity of the enzymes glutamine synthetase and L-glutamate ferredoxin oxidoreductase. *In vivo* research by the same author (Van Hezewijk 1994), in which the effects of different amounts of ammonium nitrate, ammonium sulphate, urea and potassium nitrate were investigated in pot experiments, revealed that only potassium nitrate was not capable of reducing *Orobanche crenata* infestation. Urea was slightly less effective than the two ammonium fertilizers. Relatively low amounts of fertilizer were already sufficient to induce the maximum effect. The effect could be enhanced by the use of nitrification inhibitors. Field experiments could only partly confirm these results (Van Hezewijk et al. 1991b, Van Hezewijk 1994). The practical importance of the use of ammonium fertilizers (either with or without nitrification inhibitors) as a control measure of *Orobanche crenata* is limited, especially in highly infested fields.

Organic manures are also known to reduce the infestation of *Orobanche* spp. and increase crop yields. The effect may be caused by the stimulative effect on biological activity of the soil, reducing the viability of the seeds and limiting the stimulative effect of root exudates on the germination of the seeds.

## 2.7 Control during the parasitic (reproduction) phase (Table 3)

### 2.7.1 Physical methods

**Weeding:** Hand weeding of emerged broomrape shoots is the universally recommended method of control. However, it is very expensive, causes injury to crop plants and is ineffective in terms of economic returns on the labour investment, since the parasitic weeds already inflict damage before emergence. It does, however, prevent seed production and therefore a further increase of the seed bank.

Special tools for hand weeding in tobacco (the so-called "spear" or "mini-spear plus leaf pusher") have been developed and subsequently accepted by the farmers in India (Krishnamurthy and Nagarajan 1991a, Krishnamurthy and Raju 1994b).

Once a week hand-picking of broomrape in a tobacco crop before seed ripening reduced broomrape population from 366 m<sup>-2</sup> to 13 m<sup>-2</sup> after 7 years in India (Vyas 1966). Although pulling is impossible in case of dense infestations, it should, however, be practical to pull out sparse infestations, as in crop mixtures and the

Table 3. Possible control methods during the parasitic and reproductive phase of the growth cycle of *Orobanche*.

Effect	Methods			
	Physical	Chemical	Biological	Other cultural
Killing of tubercle or shoot before emergence		Systemic herbicides, Fumigants		
Killing of shoot after emergence	Weeding, Flaming	Herbicides, Plant oils, Kerosene, Diesel oil, Ethephon	Insects, Fungi	
Improving crop tolerance			Tolerant cultivars	Fertilization, Manuring

residual populations after the use of other control measures like herbicides, fertilizers, resistant varieties, etc. (Ramaiah 1984).

Cultivation between rows is sometimes also feasible, but has little positive effect, because the broomrapes develop very close to the crop plants.

**Flaming:** Eplee et al. (1994a) reported that local infections of *O. ramosa* can be destroyed by surface incineration.

### 2.7.2 Chemical methods

Most of the research efforts in controlling broomrape have been concentrated on finding chemicals that can be used effectively in the field. In this section, we only discuss chemical control of tubercles or shoots (either before or after emergence). Two separate approaches can be observed: the *Orobanche* shoots can be attacked directly by the chemical or can be controlled through treatment of the host plant. Hundreds of promising herbicides have been tested. Some results are recently summarized by Eplee and Norris 1995. Necessarily, we only discuss a selection of the most promising ones.

**Pre- and post-emergence herbicides:** Herbicides such as imazethapyr, imazapyr, imazaquin and chlorsulfuron can be applied after germination of the crop but before crop emergence and can reduce infestation without damaging the crop. The efficiency, however, is not always satisfactory, partly because the effects strongly depend on environmental conditions. Some of these herbicides can also be used

after crop emergence (see below). Chlorsulfuron at the rate of 10 g/ha reduced broomrape emergence by 88% in tomato (Kotoula-Syka and Elephtherohorinos 1991).

Zahran et al. (1989) observed that the control of broomrape was best with dazomet at 25 kg/ha incorporated pre-sowing. Yield increase of faba beans was 37% as a result of the control.

Jacobsohn et al. (1987) reported that in heavy clay soil 1000-2000 l vapam (metham-sodium) per ha gave very good control of a heavy infestation of *Orobanche aegyptiaca* and considerable yield increase in tomato. Also, the germination of *Orobanche aegyptiaca*, *O. ramosa* subsp. *mutelii*, *O. crenata* and *O. cernua* was completely inhibited by vapam at 1000 l/ha injected into the sprinkler irrigation system.

**Systemic herbicides:** Systemic herbicides such as glyphosate and the diazin MH can be very effective in controlling *Orobanche* either before or after emergence of the shoots. Glyphosate is sprayed on the host crop and is translocated to the parasite to kill it. Its phytotoxicity is the most limiting factor. Host crops which tolerate glyphosate are vetch, broad bean, carrot, cabbage and celery. Tomato, eggplant and pea are extremely sensitive to the chemical (Jacobsohn and Levy 1986). Post-emergence sprays of glyphosate at 0.064 kg/ha and imazaquin at 0.09 kg/ha effectively controlled *O. crenata* in faba bean fields (Zahran et al. 1988). Castejon-Muñoz et al. (1990) reported that glyphosate at 0.02 - 0.04 kg/ha at 12 - 14 days interval controlled more than 80% of *Orobanche cernua* in sunflower plots. Halila (1988) observed that 60 ml glyphosate in 500 l of water per ha almost completely eliminated *Orobanche crenata* in broad bean and field bean plots. Sauerborn et al. (1989b) reported that *Orobanche crenata* and *Orobanche aegyptiaca* were controlled completely due to application of 80 g glyphosate per ha and 10 g imazaquin per ha, when broomrape attachments to the roots of *Vicia faba* were in the tubercle stage. Lolas (1994) noticed that glyphosate and sulfosate each at 0.2 + 0.3 kg active ingredient per ha and imazaquin at 0.01 + 0.10 kg active ingredient per ha when applied foliar at 40 days and 60 days after transplanting gave excellent control of *O. ramosa* with no adverse effect on tobacco growth or yield. However, 9% formulation of glyphosate damaged the crop.

In some cases low doses of glyphosate are effectively used in association with additional fertilizer (García-Torres 1994).

García-Torres et al. (1994) noticed that imazethapyr (20 - 40 g/ha), imazapyr (12.5 - 25 g/ha) and chlorsulfuron (4 - 6 g/ha) controlled *Orobanche cernua* efficiently without crop injury and the sunflower seed yields were generally similar to the non-infested checks. Imazaquin (20 - 40 g/ha), trisulfuron (4 g/ha),

primisulfuron (3 g/ha), acetochlor (4.4 kg/ha) and metazachlor (2 kg/ha) were less effective. Imazamethabenz (200 - 600 g/ha) and metochlor (3.3 kg/ha) were completely ineffective.

Although it does not inhibit *Orobanche* germination in vitro (Cézard 1973b), maleic hydrazide (MH) has a strong effect on *Orobanche* development. In tobacco infected with *Orobanche ramosa*, Lolas (1986) had 80% fewer *Orobanche* plants when 0.7 kg of MH per ha was applied twice (i.e. 40 and 60 days after transplanting of the tobacco). This led to a considerable yield increase, similar to the positive effect of glyphosate even though fewer *Orobanche* shoots were killed than in the case of glyphosate. Later research by the same author (Lolas 1994) indicated moderate to good control of *O. ramosa* by MH at 0.45 + 0.41 kg active ingredient per ha. Also an infection of *O. aegyptiaca* in tobacco or watermelon can be controlled by MH (Cubero and Moreno 1979). In Bulgaria, MH applied at the beginning of bud formation assured an almost complete destruction of *Orobanche* in fields which were earlier disinfected with metham-sodium (Lubenov 1973). Jeleu (1988) reported that maleic hydrazide applied to tobacco 40 days after planting controlled *Orobanche* spp. by translocating basipetally and inhibiting meristematic cell division. In contrast, Kasasian (1973b) reported only partial control of *Orobanche crenata* with MH.

Torres (1987) suggested that three directed applications of ethephon at 0.2 and 0.4 kg/ha on *Orobanche ramosa* in late sown black tobacco controlled the parasite by inhibiting flower formation.

Using genetic engineering to create chemical-resistant crops may help to obtain effective control of the weed without the negative side-effects on the crop (Joel et al. 1992b, Gressel et al. 1994, see also earlier).

**Oils:** Post-emergence swabbing of oil on young *Orobanche cernua* shoots will kill the weed without affecting the host plant. The method is only effective when carried out before flowering of the *Orobanche cernua* and is very labour intensive, as all shoots must be treated individually because the oils should not come into contact with host plants. The treatment has to be repeated when new shoots emerge. Both plant oils and mineral oils have been tested for their effectiveness.

Plant oils (castor oil, cotton seed oil, linseed oil, rice bean oil, soybean oil, eucalyptus oil, pongamia oil, tobacco seed oil, coconut oil, gingelly oil, groundnut oil, mustard oil, neem oil, safflower oil, sunflower oil, and palm oil) killed the parasite by suffocation when the oils were applied on the top of young broomrape shoots (Krishnamurthy and Chari 1991, Krishnamurthy and Nagarajan 1991b, Krishnamurthy 1992). Not all plant oils were equally effective, but they have the advantage of not being phytotoxic to the host plant.

Swabbing of *Orobanche cernua* shoots with mineral (kerosene) oil resulted in

85% mortality of *Orobanche cernua* but at the same time retarded growth of tobacco (Krishnamurthy et al. 1976) or caused the tobacco leaves to be scorched (Krishnamurthy and Nagarajan 1991b). Diesel oil and thinner, mineral oils had similar effects. Swabbing kerosene or diesel oil on young broomrape (*Orobanche papaveris*) shoots in a opium poppy crop resulted in a 37.5 and 61.0% mortality of the parasite, respectively (Ramanathan 1985). A combination of diesel oil and allyl alcohol was even more effective, especially at high temperatures (Ramanathan 1985).

### 2.7.3 Biological methods

Biological control of broomrape has been attempted by means of the insect *Phytomyza orobanchia* Kalt (Diptera, Agromyzidae), of which the adults feed on the inflorescence of the parasite (Nemli and Giray 1983). This insect is the most important pest of *Orobanche*. It is oligophagous and feeds only on broomrapes. The larvae feed on the stem as well as on the developing fruits. In the Ukraine, complete elimination of *Orobanche cernua* in tomato crops and increases in cabbage yields were obtained when this fly was introduced (case cited in Hosmani et al. 1993). Van Hezewijk (1994) reports that releasing the fly in the field when *Orobanche* was just emerging reduced the infestation to a minimum in the former USSR. In Hungary, introduction of *Phytomyza orobanchia* in sunflower fields controlled *Orobanche* by 69% (Horvath 1987). In Turkey, *P. orobanchia* destroyed the *Orobanche* capsule to 94% (Giray and Nemli 1983). The fly can sense *Orobanche* over a distance of 3 km and can spend 3 years in diapause in the hibernating stage, thus surviving temporary absence of the host. Its population, however, is threatened by parasitic wasps.

In many infested areas, the fly is reducing the seed production considerably, but for proper control it is necessary to increase the population artificially (Hammad et al. 1967, Linke et al. 1990).

In certain soils, the fungus *Fusarium solani* has been detected as the main factor suppressing the growth of *O. ramosa* in tomato (Gold et al. 1979, Hodosy 1981). Also *Fusarium orobanche* is effective against *Orobanche* (Kott 1969b) and could be used in the development of bioherbicides to control the parasitic weeds in their vegetative phase. Inoculation of the fungus *Fusarium oxysporum* f.sp. *orthoceras* in the field resulted in 90% control of *Orobanche* in sunflower (Bedi and Donchev 1991, Bedi 1994, Sauerborn et al. 1994) or tomato (Hodosy 1981), whereas infection of watermelon by *Orobanche aegyptiaca* could be reduced by 90 - 97% (Panchenko 1974) and this fungus has potential as a mycoherbicide. It was developed into "Product F" in the former USSR. Other reports (e.g. Timchenko and Dovgal 1972) on control of *O. ramosa* by this fungus were less optimistic: in tomato and cabbage *O. ramosa* was reduced by 44 - 67%. Further *Fusarium*

species with positive effects on *Orobanche* control include *F. gibbosum* and *F. lateritium* (Taslakh'Yan and Grigoryan 1978). *Fusarium lateritium* has given promising results for control of *O. ramosa* and *O. muteli*, both affecting tobacco (Bozoukov and Kouzmanova 1994).

Promising results on biological control have also been obtained with the fungi *Rhizoctonia solani* (on *O. ramosa*) and *Alternaria* spp. and *Sclerotinia* sp. on *O. crenata* (Duafala et al. 1976, Al-Menoufi 1986). Linke et al. (1992) isolated as many as 16 fungus species of the genera *Alternaria*, *Fusarium* and *Ulocladium* from *Orobanche* plants as potential biological control agents. The same authors observed 100% control of *Orobanche* by the fungus *Ulocladium atrum*, provided the relative humidity was high.

Relatively high soil humidity and soil temperatures are required for the development of fungi. More research is needed to develop a reliable biological method.

#### 2.7.4 Other cultural methods

Fertilization and manuring may improve crop tolerance by enhancing crop vigour.

In addition to the use of resistant cultivars, in which case no infection will occur, the use of tolerant cultivars may be considered. There is genetic variation in the sensitivity to infection by *Orobanche crenata* (e.g. Abdalla and Darwish 1994) but more research is needed to elucidate the underlying mechanisms.

### 2.8 Integrated control

Lolas (1986) stated that there are no single effective, economical and practicable remedial measures for the parasite. There are effective measures, but they are too expensive to be used in most countries where *Orobanche* is a problem. Other methods are effective (such as fumigant or herbicide application) but are hazardous to the environment or reduce crop growth too much. Some methods (such as biological control or control by delayed sowing or fertilization) have positive effects but control is far from complete. Integration of methods could be beneficial and has received some attention in research.

Kukula and Masri (1984) suggested a combination of removal of seeds from the tilth by tillage, control in the germination phase by adequate fertilization and control in the parasitic and reproductive phase by glyphosate.

Ramanathan (1985) stated that complete eradication of *Orobanche papaveris* would be realized in India in 3 - 4 years through a feasible combination of luring with hosts or non-hosts (by sowing of a mixture of trap and catch crops in July and ploughing under in September), chemical control of emerged shoots (by

swabbing a mixture of diesel oil and allyl alcohol on *Orobanche cernua* spikes in the period February to April), and physical control (by burning dry spikes with seeds standing in the field in April) before the following trap or catch crop was sown.

Linke and Saxena (1991) and Saxena et al. (1994) described integrated systems of *Orobanche* control in the Mediterranean areas of West Africa and North Africa making use of solarization to kill seeds, early maturing and less infected cultivars and delayed sowing (to reduce germination and tubercle formation), low concentrations of herbicides (glyphosate or imazaquin to kill the weed in its early stages of development), and hand weeding to kill emerged shoots that escaped, before seed shedding could take place. Hand weeding was feasible because the number of shoots was already strongly reduced by the other treatments.

Nassib et al. (1984) described integrated systems for control in faba bean in Egypt: seeds were kept away from the tilth through zero tillage; tolerant or resistant cultivars, planted in high densities, further decreased the seed reserves and/or reduced damage to the crop and high levels of fertilizer reduced infection and increased tolerance of the crop.

Pieterse et al. (1994) indicated that in faba bean crops in Syria and Egypt the best results were obtained with a combination of slightly delayed sowing and low concentrations of imidazolinone herbicides and/or glyphosate. These authors also indicated the importance of tolerant or resistant lines of faba bean (mainly derived from the cultivar Giza 402) and the potential of biological agents.

Although the choice of method of control depends on many agronomic and economic considerations (such as size and population dynamics of the seed bank, cropping system, weather and climate, costs of application, damage to the crop in relation to possible effect of successful control, and sensitivity to risk), some general statements about the best combination of methods can be made.

Low-cost integrated control of *Orobanche* should include removal of seeds from the seed bank, either by physical methods (tillage) or by biological techniques (growing of catch crops, trap crops). It should also include prevention of germination or killing of structures before attachment by chemicals. Cultural practices, such as sowing time, irrigation and fertilization, should be adjusted in such a way that they contribute to the control of the parasite and the tolerance of the crop, provided yield losses caused by these adjustments are acceptable. The few shoots that still emerge should be controlled by individual treatment with herbicides, oils or mechanical weeding before seed set. Emphasis should be on the development of resistant cultivars of crops prevailing in the crop rotation.

## 2.9 Conclusion

This review highlights the complexity of the parasite's etiology and hints at

management strategies to be adopted in controlling it. Physical methods are useful to prevent any *Orobanch*e development but are expensive or not feasible except for the removal of seeds from the tilth. Chemical and cultural methods can reduce infection through various effects, but they are often too expensive, control may be incomplete or the treatment may cause a yield reduction of the crop. Some biological methods are promising but still need more research. However, the option of improving durable resistance of crops is interesting.

In many cases a combination of methods may prove to be most efficient. The integration of cultural and chemical approaches especially may further help to control broomrape effectively, even though it is very costly.

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## Chapter 3



## Chapter 3

### FIELD OBSERVATIONS ON INTERACTIONS BETWEEN *OROBANCHE CERNUA* LOEFL. AND BIDI TOBACCO IN NIPANI, INDIA

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#### Summary

Interactions between *Orobanche cernua* and bidi tobacco were recorded in naturally infested tobacco fields at Nipani, India. At harvest, most broomrapes occurred in clusters of 4 - 9 spikes per tobacco plant. The effect of broomrapes became visible at 50 days after transplanting tobacco and were mainly caused by a reduction of the growth rate of the tobacco plant and not by shortening the growth period. The tobacco crop grew exponentially until 60 days and thereafter linearly in the period 60 - 140 days after transplanting. From 140 days onwards the growth of the crop became truncated. The reduction in tobacco yields depended on the number of broomrapes per tobacco plant. Individual broomrape spikes were lower in dry weight when more spikes occurred per tobacco plant.

#### 3.1 Introduction

Interactions between organisms in nature are the rule rather than the exception. When the organisms live in or on each other with some degree of permanency and interact nutritionally, such interactions are termed as 'symbiosis'; in parasitism one organism benefits to the detriment of the other members of the association (Douglas, 1994). *Orobanche cernua* Loefl. is a scapigerous, herbaceous, insidious root parasite causing severe damage to Solanaceous crops such as tobacco, tomato, eggplant and potato. Tobacco is seriously affected by *Orobanche cernua* in India. It parasitizes all types of tobacco alike, disrupting the physiological and

metabolic processes in the host, resulting in reduced crop growth, wilting and ribbed appearance of leaves, and chlorosis (Krishnamurthy, 1991).

Broomrapes reproduce by means of seeds that are dark brown, oval and very small (0.35 × 0.25 mm). They have an enormous seed production potential. Moreover, seeds remain viable in the soil for up to 20 years (Puzzilli, 1983); they even remain viable after passing through the rumen of sheep (Jacobsohn et al., 1987) or the gut system of earthworms (Ter Borg et al., 1996). Seeds germinate only under the influence of root exudates up to a distance of a few millimetres from the host plant (Kadry and Tewfic, 1956). After germination, the germ tube penetrates the root tissue, then makes a connection with the vascular tissues in the root and a haustorium is developed on the host plant. The haustorium is the physiological and morphological bridge between the host and the parasite but its function is more than a conduit (Kuijt, 1991). The vascular tissue of the host root becomes connected with the tubercle (a bulb-shaped organ). Then, the tubercle produces numerous root-like organs called 'crown roots'; these crown roots attack other host roots on which they produce secondary haustoria. Later on buds are produced by the tubercle which subsequently form the main axis and the inflorescence (see also Hosmani et al., 1993; Dhanapal and Struik, 1996b). Wind, water, animals and men may disseminate such seeds (Berner et al., 1994).

The life cycle of broomrape is ideally suited for an obligate mode of parasitism. In India, *Orobanche cernua* seeds germinate during the second week after planting tobacco, the germ tube infects the host roots during the third week and later tubercles develop into below-ground sprouts until the fifth week under favourable conditions. The parasitic shoots start to emerge from the sixth week onwards. Flowering is completed by the eighth or ninth week, drying of the stem by the tenth week and dehiscence of the capsule by the eleventh or twelfth week. Thus, in less than three months after planting tobacco the life cycle of the parasite is completed (Krishnamurthy et al., 1977).

A thorough understanding of the biology of *Orobanche cernua* Loefl. in relation to the growth of the tobacco crop will help us to predict the limitations and benefits of various control measures or may hint at effective, safe and economical methods of control. Detailed studies on various fundamental and applied aspects of *Orobanche cernua* on tobacco in India are scarce. Therefore, field studies were conducted in a naturally infested tobacco field to describe above-ground growth and development of the parasite and its effect on growth of bidi tobacco.

### 3.2 Materials and Methods

Growth and development of *Orobanche cernua* were studied in a tobacco (*Nicotiana tabacum* L.) field naturally infested with *Orobanche cernua* at the

Agricultural Research Station, Nipani, India during 1994-1995 in the late kharif (spring; rainy period) and rabi (winter; cool, dry) seasons. Bidi tobacco was grown at the research station since 30 years.

For above-ground or post-emergence life cycle studies of *Orobanche cernua*, 50 freshly emerged *Orobanche cernua* shoots were labelled in a bidi tobacco crop and observations were done periodically in the season 1994-95. Observations on *Orobanche* included days required to emerge above ground, days taken to complete flowering, days taken for stem drying and dehiscence of the capsules, density and dry weight at harvest. Observations on tobacco included plant height, number of leaves and dry weight of leaves (g/plant) at final harvest.

In another post-emergence study during 1994-95, 30 freshly emerged *Orobanche cernua* spikes were labelled in a bidi tobacco crop and observations were recorded at 10 days interval from 30 days after transplanting until harvest (185 days after transplanting). Data recorded included number and height of broomrape spikes and plant height of tobacco. The effect of *Orobanche cernua* on the bidi tobacco crop was recorded periodically in the morning hours using the following visual scoring:

<u>Score</u>	<u>Effect on tobacco crop</u>
1	none
2	wilting, drooping and ribbing of leaves
3	stunted growth

### 3.3 Results

#### 3.3.1 Rate of development of *Orobanche cernua*

Studies on the life cycle of *Orobanche cernua* in bidi tobacco during 1994-95 (Table 1) revealed that the spikes started emerging above-ground from 55 - 60 days after transplanting its host crop. This is much later than usual, because of heavy rainfall before transplanting the tobacco crop. Until 55 - 60 days after transplanting tobacco, different phases of development of the parasite occurred below-ground. Flowering was completed by 65 - 70 days after transplanting tobacco. Stem drying and dehiscence of capsules was completed by 95 - 100 days after transplanting. The life cycle of the parasite was completed in about 100 - 110 days after planting tobacco.

#### 3.3.2 Growth of *Orobanche cernua* and tobacco

The maximum height of *Orobanche cernua* was 29.4 cm at final harvest. Most broomrapes were found in the range of 15.1 to 20.0 cm (Fig. 1). Few above-ground broomrapes grew to less than 10 cm at final harvest.

At final harvest, tobacco plants supported several broomrape spikes. The major

Table 1. Rate of development of *Orobancha cernua* in bidi tobacco.

Phase	Onset of phase (DAT*)	Time required to complete phase from previous phase (Days)
Pre-germination phases		till 55
Emergence	55-60	05
Flowering	65-70	10
Stem drying/dehiscence of capsule	95-100	30
Completion of life cycle	100-110	10

\* Days after transplanting

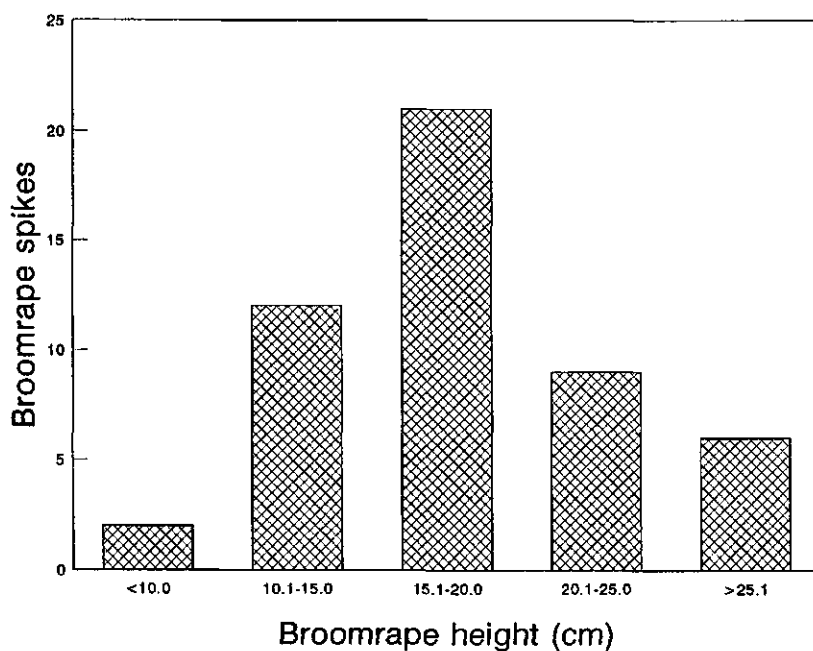


Fig. 1. Frequency distribution of broomrape height at final harvest.

part of the broomrape plants occurred on tobacco plants carrying 4 - 9 spikes per tobacco plant (Fig. 2).

The development over time of the height of broomrape and tobacco is depicted in Figure 3 and analysed using the Gompertz's function (Table 2; Hunt, 1982). The tobacco crop grew exponentially till 60 days and thereafter linearly in the period 60 - 140 days after transplanting. Later on, the growth of the crop became truncated. The broomrape height was levelled off after 140 days after transplanting of the tobacco.

Observations on the wilting pattern of affected plants during morning hours revealed that wilting of leaves commenced early during the day; leaves returned to normal condition during night and to near-normal condition by early morning. During mid-day the tobacco plants experienced water deficit due to *Orobancha cernua* infestation. These observations were visually scored as score 1, score 2 and score 3. Figure 4 illustrates the effect of broomrapes on the tobacco plant height and Table 2 gives the values of the coefficients in the Gompertz and Sigmoidal functions. The tobacco plant height was reduced at score 2 and even more so at score 3 with lower asymptotic values at score 2 and 3 (Table 2). The effect of broomrapes was already visible at 50 days after transplanting tobacco. The relative growth rate of the tobacco plant derived from Gompertz's parameters

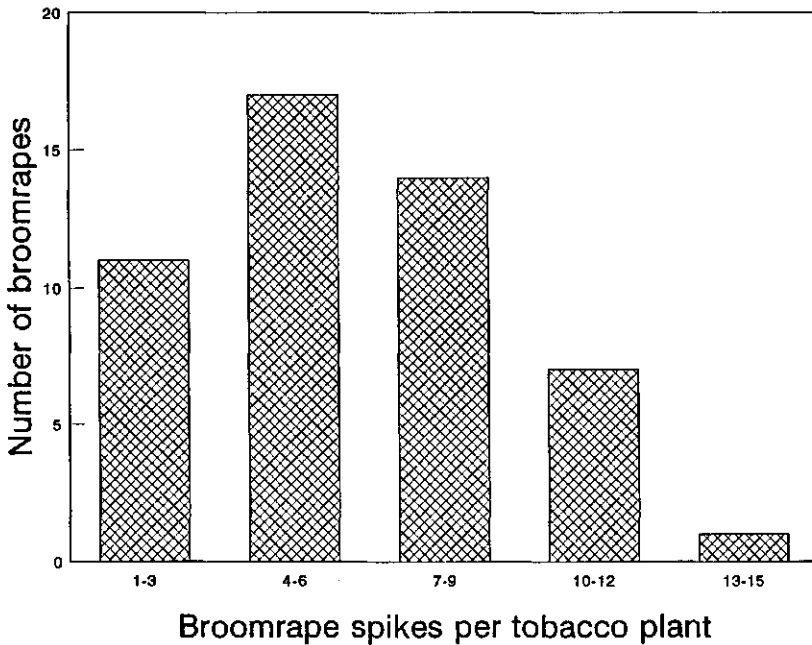
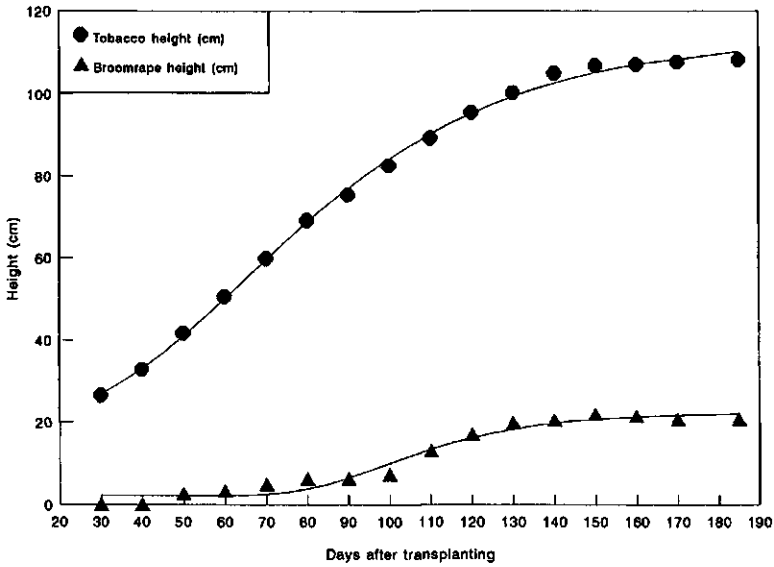
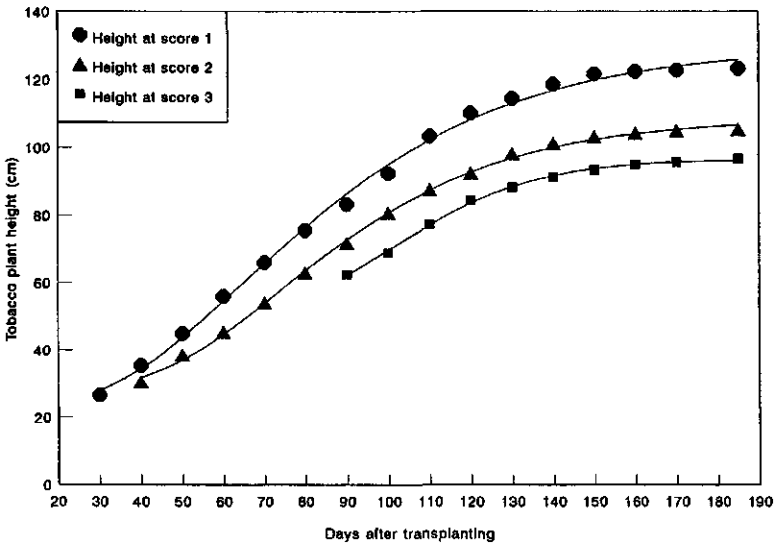


Fig. 2. Frequency distribution of broomrape population per tobacco plant at maturity.



Data points are averaged over 30 observations and fitted by Gompertz function (Hunt, 1982);  $r^2$  adj. = 0.998 and 0.957 for tobacco height and broomrape height respectively.

Fig. 3. Broomrape height and tobacco height over time.



Data points are averaged over 30 observations and fitted by Gompertz function (score 1 & 2) and sigmoidal function (score 3)(Hunt, 1982);  $r^2$  adj. = 0.997, 0.998 and 0.998 for score 1, 2 and 3 resp. Score 1: no effect; Score 2: wilting, drooping and ribbing of leaves; Score 3: stunted growth

Fig. 4. Effect of broomrape on tobacco plant height over time.

Table 2. Values of the parameters of Gompertz and Sigmoidal functions fitted to curves in figures 3, 4 and 6 (Hunt, 1982). Sample size n=30; s.e. = standard error.

		Parameters				r <sup>2</sup> adj.
		a	b	c	d	
<u>Figure 3</u>						
Tobacco*	mean	19.6	94.2	0.027	64.30	0.998
	s.e.	3.6	4.7	0.002	2.64	
Broomrape*	mean	2.2	20.1	0.048	98.93	0.957
	s.e.	0.8	1.7	0.012	3.57	
<u>Figure 4</u>						
Score 1*	mean	19.9	110.0	0.028	65.24	0.997
	s.e.	4.8	6.3	0.002	3.01	
Score 2*	mean	27.5	81.3	0.032	73.60	0.998
	s.e.	2.1	2.8	0.002	1.62	
Score 3**	mean	42.4	54.3	1.998	1.76	0.998
	s.e.	7.1	7.5	0.469	0.20	
<u>Figure 6</u>						
0 - 5 spikes/plant*	mean	24.5	95.2	0.029	69.90	0.997
	s.e.	3.4	4.7	0.002	2.43	
6 - 10 spikes/plant**	mean	43.4	64.2	102.090	18.26	0.995
	s.e.	13.1	14.0	7.653	3.45	
11 - 15 spikes/plant**	mean	-313.7	412.1	4.808	35.96	0.997
	s.e.	1988.9	1991.0	219.300	13.75	

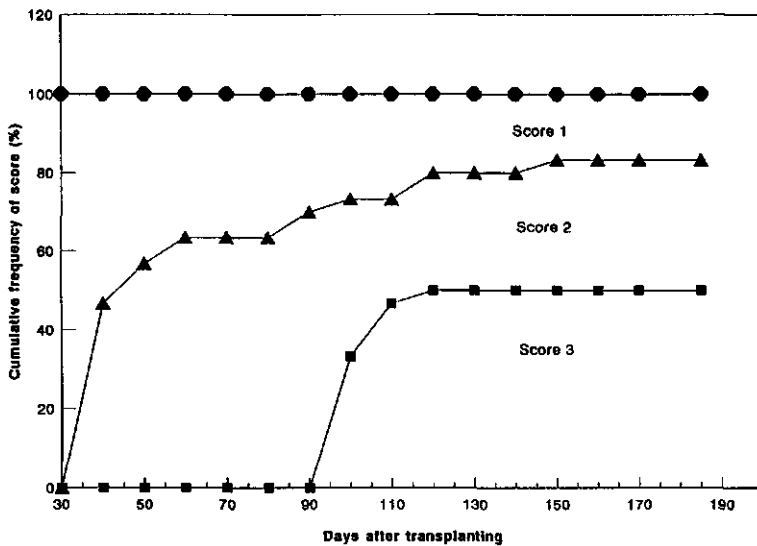
\* Gompertz function:  $Y = a + b * \exp(-\exp(-c * (x - d)))$

\*\* Sigmoidal function:  $Y = a + b / (1 + \exp(-(x - c) / d))$

a: starting size; b: asymptote (maximum value); c: rate of increase; d: measure of inflection point  
r<sup>2</sup> adj.: adjusted regression coefficient

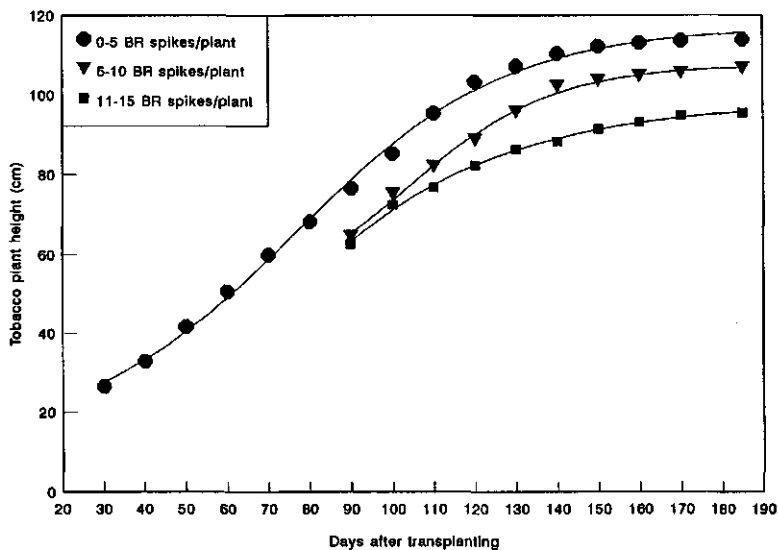
(not shown) decreased rapidly with score 2 and 3. Yet, the duration of plant height increase was not different for the three classes of tobacco plants.

The change in time of the relative frequencies of the three classes is shown in Figure 5. The number of plants with score 2 increased at the expense of plants with score 1. Later the number of plants with score 3 increased mainly at the



Score 1: no effect  
 Score 2: wilting, drooping and ribbing of leaves  
 Score 3: stunted growth

Fig. 5. Effect of broomrape population on the tobacco plants.



Data points are averaged over 30 observations and fitted by Gompertz function (0-5) and Sigmoidal function (6-10 & 11-15) (Hunt, 1982);  $r^2$  adj. = 0.997, 0.995 & 0.997 for broomrape spikes of 0-5, 6-10 and 11-15 resp.; 0-5, 6-10 & 11-15: Broomrape spikes per tobacco plant

Fig. 6. Effect of the number of broomrape spikes per plant on the tobacco plant height over time.



expense of plants with score 2. After 110 days of transplanting 50% tobacco plants had score 3, 30% score 2 and 20% score 1.

The effect of the number of broomrape spikes per tobacco plant on its height is depicted in Figure 6 and analysed using Gompertz and Sigmoidal functions (Table 2). Tobacco height was more reduced when there were more broomrape spikes per tobacco plant. The reduction in tobacco plant height was 22 - 27% with 11 - 15 broomrape spikes per tobacco plant and 15 - 17% with 6 - 10 spikes per plant compared to a broomrape population in the range of 0 - 5 per tobacco plant at 110 - 185 days after transplanting. With 0 - 5 broomrape spikes per tobacco plant, the tobacco growth was linear in the period 60 - 140 days after transplanting. Until 60 days after transplanting tobacco plants grew exponentially. Plants affected by different numbers of broomrapes differed significantly in their growth characteristics (Table 2). The effect was mainly through a reduction of growth rate and not by shortening the growth period.

The effect of different numbers of spikes per tobacco plant on the final plant height of tobacco is illustrated in Figures 7 and 8 for the two series. They show that there is a close negative, linear relationship ( $r^2=0.507$ ;  $n=30$  and  $r^2=0.588$ ;  $n=50$ ) between number of spikes and the plant height at final harvest. The negative, linear relationship between number of spikes and leaf dry weight was even more significant (Fig. 8;  $r^2=0.825$ ;  $n=50$ ).

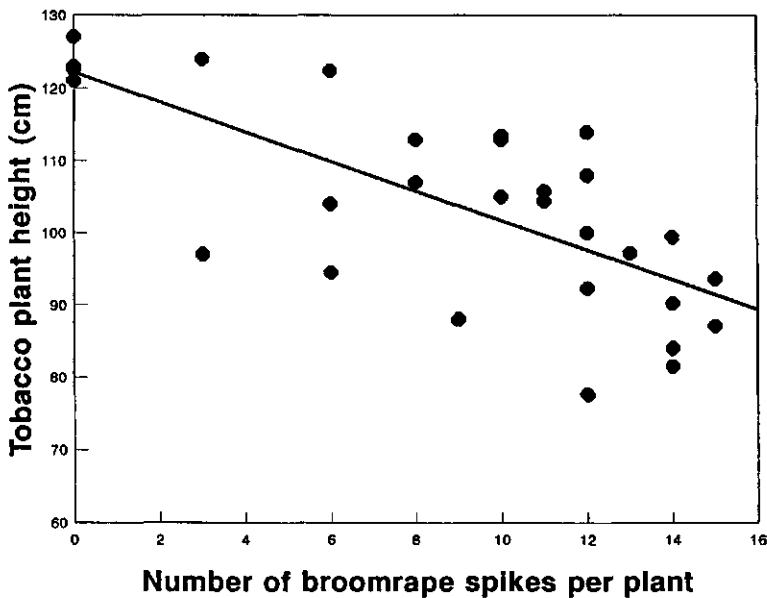


Fig. 7. Relation between number of spikes per plant and the tobacco plant height.

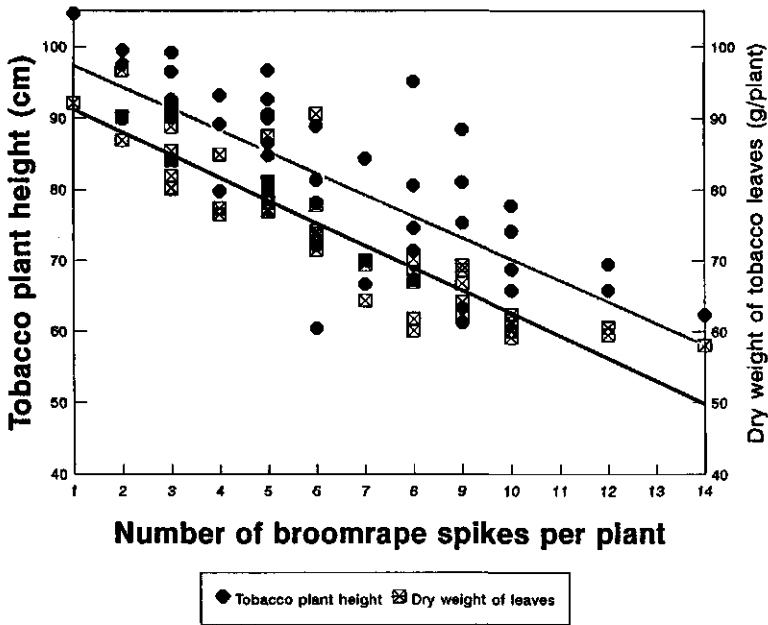


Fig. 8. Relation between broomrape spikes per plant height & leaf dry weight of tobacco.

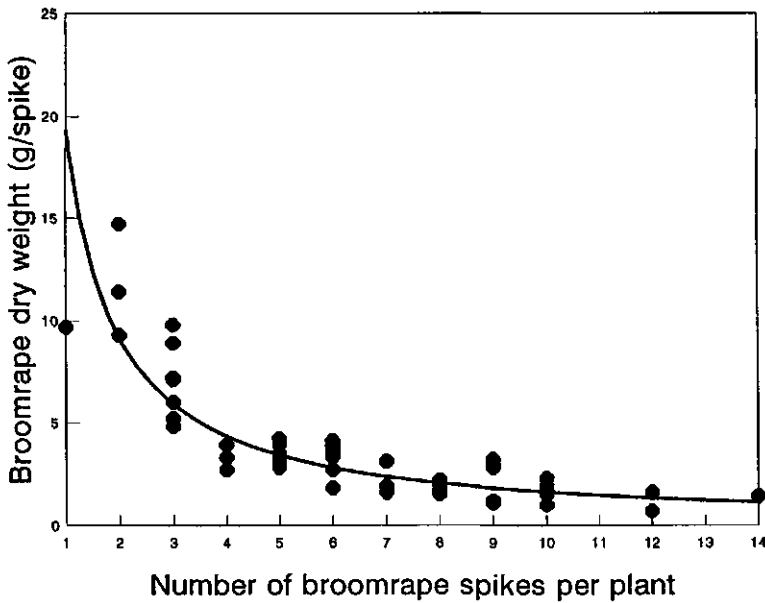


Fig. 9. Relation between broomrape spikes per plant and dry weight of broomrape at harvest.

Broomrape number per tobacco plant had no effect on the average spike height of broomrape ( $r^2=0.020$ ;  $n=50$ ). However, the exponential, negative relation between number of spikes per tobacco plant and the average dry weight of the spikes was highly significant (Fig. 9;  $r^2=0.740$ ;  $n=50$ ).

### 3.4 Discussion

One of the important features and diagnostic symptoms of *Orobanchae cernua* infection is wilting and ribbing of tobacco leaves which ultimately results in stunted growth of the host plant. Our data indicated the reduction in tobacco plant height and dry weight of leaves depending upon the severity of infestation of the parasite. Similar observations were made by Krishnamurthy et al. (1977). Transpiration of *Orobanchae* spikes, however, is small and therefore they hardly withdraw water from their hosts (Ehleringer and Marshall, 1995). The wilting of faba bean plants infected with *Orobanchae crenata* was attributed to carbohydrate starvation of the host roots and not to the direct effect of removal of water (Whitney, 1972).

Crop growth was reduced by *Orobanchae cernua* attack. The reduction in plant height of tobacco depended upon number of *Orobanchae cernua* spikes present per tobacco plant. Dhanapal and Struik (1996a) observed that *Orobanchae cernua* reduced plant height by 19%, the number of leaves by 32% and the dry weight of leaves by 39%. In the present study, dry weight of leaves of tobacco was reduced by 41% at 11 - 15 broomrape spikes and by 23% at 6 - 10 spikes per tobacco plant as compared to a broomrape density of 0 - 5 per tobacco plant.

The growth rate of a plant is not only determined by the rate of assimilation but also by the partitioning of the resources (Poorter and Remkes, 1990); allocation of resources to leaf growth and growth form of leaves may be more determinant for the rate of the plant growth than net assimilation rate. The presence of a parasite can radically alter the partitioning within the host, in turn it affects the host growth rate; Ernst (1986) observed increased root:shoot ratio in tobacco plants infested with *Orobanchae ramosa*. The increased host root:shoot ratio may improve the durability of the association in the face of adverse environmental conditions; a larger root system will explore a greater volume of soil and thereby be of benefit during a period of water deficit (Dörr et al., 1977). Ter Borg and Van Ast (1991) suggested that there may be a positive association between host root weight and parasite weight. The point deserves further attention with respect to the tobacco-broomrape system.

Emergence of *Orobanchae* above-ground depends on the point of attachment in the rhizosphere (depth in the soil), soil moisture content and the quantity of stored carbohydrates in the parasite. No information was collected with respect to the number of non-emerged spikes and it cannot be estimated since no information is

available in the literature on the relation between the number of above-ground and non-emerged spikes. Moreover the relation may be disturbed sometimes, when more spikes grow from one tubercle. If there are many attachments below-ground then there may be a lower proportion of emerged *Orobanche cernua* spikes above-ground, due to competition. Competition may also have effected the negative relation between spike numbers and their average dry weight; it was remarkable to see that spike height was not affected. A further study, including digging of root systems is required to answer these questions. The data support the statements by Graves (1995): *Orobanche* is a holoparasite entirely dependent on the host for resources; it may have a deleterious effect on host performance and its effect is often ameliorated by a low growth rate; a combination of parasite size, growth rate and metabolic activity of the parasite and degree of dependency on the host for resources and the growth stage of the host will decide the magnitude of the effect on the host, and any one of these separately will be a poor predictor of the intensity of the host-parasite interaction.

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## Chapter 4

## Chapter 4

### BROOMRAPE CONTROL BEFORE ATTACHMENT TO HOST THROUGH CHEMICALLY OR BIOLOGICALLY MANIPULATING BROOMRAPE GERMINATION

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#### Summary

The germination phase of *Orobancha cernua* Loefl. is a critical period. The seed bank of the parasite can be reduced by stimulating the germination through chemicals in the absence of hosts or through natural stimulants by exposing seeds to trap crops. Seven series of laboratory experiments were conducted to investigate different methods of testing and to study the effect of several chemicals, root exudates of germinating crop seeds, and their interactions on *Orobancha cernua*. Best results were obtained when germination was tested in the presence of seedlings of host plants under glasshouse conditions. GR24 at 0.1 and 1.0 ppm was found most effective in stimulating the germination of the seeds followed by gibberellic acid at 10 and 20 ppm. Without addition of chemicals, trap crops strongly increased germination. Especially greengram and sunhemp were effective. The effects of (concentrations of) chemicals and trap crops interacted. The positive effect of trap crops was observed even when there was already a strong stimulation by GR24 at 1.0 ppm, but effects of trap crops were smaller at 0.1 ppm GR24. Within one host plant species, no differences between cultivars in their effect on *Orobancha* germination could be detected.

#### 4.1 Introduction

*Orobancha cernua* Loefl. is a holoparasite and the most pernicious parasitic species of the genus *Orobancha* in the tobacco crop in India; it is a prolific seed producer and seeds remain viable in the soil for a long period. In their seed stage broomrapes are quite resistant to most of the weed control measures, except to soil treatment, such as fumigation and solarization (Foy et al., 1989). Germination



can only take place in the presence of root exudates containing a germination stimulant (Chabrolin, 1938; Brown, 1946). Once broomrape seeds have germinated, they must establish contact with the host roots rapidly in order to derive nutrients and water for further growth and development. This germination phase is a critical period (Musselman and Press, 1995; Dhanapal et al., 1996 and papers cited therein). If broomrape seeds are stimulated to germinate in the absence of host plants, the parasite seedlings die because of lack of nutrition. This phenomenon could be the basis for parasitic weed control, using chemicals that stimulate germination in the absence of host plants.

Upon germination, a root-like structure called germ tube (Kadry and Tewfic, 1956) is formed. If it reaches the root of a host plant, the apex penetrates the root tissue and a haustorium is developed on the host plant. Penetration through the host epidermis involves dissolution of the middle lamella, while penetration through other tissues also employs mechanical pressure that pushes portions of cell walls aside; dissolution of cell walls could be traced in the vascular cylinder of the host roots (Joel and Losner-Goshen, 1994). Ben-Hod et al. (1993) assumed that pectinase enzymes are involved in the process of penetration. The vascular tissue of the host root becomes connected with a developing bulb-shaped organ, the 'tubercle'. Later, the tubercle produces numerous root-like organs resulting in a 'crown like' appearance; these organs are called crown roots. The crown roots attack other host roots on which they produce secondary tubercles. After the production of crown roots, buds are produced by the tubercle which will subsequently form the main axis and the inflorescence.

The predominant factor in the root exudate responsible for broomrape seed germination is host specific (Singh and Pavgi, 1975); it plays a role in host recognition (xenognosis) and therefore host-derived germination stimulants are also called 'xenognosins'; they are very labile (Boone et al., 1995). However, many non-host plants are capable of stimulating the germination of broomrape seeds (Brown et al., 1951; Singh and Pavgi, 1975; Krishnamurthy et al., 1977; Press et al., 1990). This suggests that there are several (types of) stimulants. Other xenognosins may also play a role in the attachment and subsequent development of the haustorium; this is true in case of *Striga* (Lane et al., 1994; Riopel and Timko, 1995), but for *Orobanche* spp. it is still a matter of speculation.

In recent years, considerable interest has been generated in isolating, identifying and synthesizing the chemical factors in root exudates of plants capable of stimulating broomrape seed germination. Much research has focussed on the nature of these stimulants, and some have been identified (Stewart and Press, 1990). Strigol (Cook et al., 1972) and the related compounds alectrol (Müller et al., 1992) and sorgolactone (Hauck et al., 1992) are natural stimulants. A well-known source of strigol in nature is that of the root exudates of cotton (Foy et al., 1989). Strigol

has long been the subject of many synthesis studies, and to date seven total syntheses and several partial syntheses have been reported (Zwanenburg et al., 1994). The isolation and purification of strigol in large amounts from root exudates appears impractical; an artificial synthesis of strigol is necessary to obtain enough compounds for commercial use.

Johnson et al. (1981) stated that the strigol analogue GR24 has the highest activity of all GR compounds but cannot be used on a larger scale due to its limited availability and high cost; GR24 compound is active at 0.1 to 1.0 ppm concentrations on *Orobanchae* seed germination (Spelce and Musselman, 1981; Parker and Riches, 1993). Jacobsohn et al. (1988) observed over 90% germination of broomrape conditioned in water and then stimulated to germinate by GR24. GR7, GR24, GR28, and GR41 are most active at 0.1 to 1.0 ppm on *Orobanchae* seed germination (Saghir, 1979; Spelce and Musselman, 1981; Saghir et al., 1983; Saghir, 1986); these compounds tend to inhibit germination at higher concentrations (Saghir, 1979).

Gibberellic acid ( $GA_3$ ) induced germination of *Orobanchae ramosa* seeds (Al-Menoufi, 1986). Possible stimulating effects on germination have also reported for ethylene (Jain, 1987), cytokinins (Edwards et al., 1976; Strelyaeva, 1978) and pyridoxine (Nash and Wilhelm, 1960). Similarly, numerous pesticides, chemically pure substances, nutrients and growth regulators have been tested for their efficacy on broomrape seed germination; only few of them could induce germination of broomrape seeds and the results are extremely variable and contradictory (Pieterse, 1981; Riches and Parker, 1995).

A suitable chemical that could induce a high per cent germination of broomrape seeds under field conditions is still lacking. Broomrape seedlings are most vulnerable to destruction immediately after germination but prior to their establishment on host roots. Therefore, a combination of such a stimulant with a control measure can help to reduce the problem. The present investigation aimed at developing a standard method to study germination of *Orobanchae cernua*, finding suitable chemicals and their optimal concentrations. Moreover, the effects of chemicals were compared with those of natural stimulants by exposing seeds to trap crops. This also allows to study the residual effect of chemicals after stimulation of germination by trap crops, to study the effect of different trap crops and to compare their cultivars.

#### 4.2 Materials and Methods

Seven separate series of laboratory experiments were conducted at the Department of Crop Physiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bangalore, India. In these series of experiments, different methods

of testing, the effects of several chemicals, of germinating crop seeds and their interactions on *Orobanche cernua* Loeff. were investigated.

The seeds of *Orobanche cernua* Loeff. which were used for the study, had been obtained from Mr. G.V.G. Krishnamurthy, Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh, India during 1992, 1993 and 1994. The seeds were stored in the dark at room temperature (20 - 25 °C) until use. A synthetic germination stimulant GR24 (analogue of strigol) which triggers germination of *Orobanche* seeds was obtained from Professor B. Zwanenburg (NSR-Center for Molecular Structure, Design and Synthesis, Department of Organic Chemistry, University of Nijmegen, Nijmegen, The Netherlands). The synthesis of this compound has been described by Zwanenburg et al. (1994). Gibberellic acid (GA; (3S, 3aR, 4S, 4aS, 7S, 9aR, 9bR, 12S) - 7, 12 - dihydroxy - 3 - methyl - 6 - methylene - 2 - oxoperhydro - 4a, 7 - methano - 9b, 3 - propenoazulenol [1, 2 - b] furan - 4 - carboxylic acid) from LUPIN-Agrochemicals India Pvt. Ltd.; ethephon (2 - chloroethylphosphonic acid) was obtained from Rhone Poulenc Agro B.V., The Netherlands; naphthalene acetic acid (NAA; 2 - (1 - naphthyl) acetic acid) from SISCO Research Laboratory Pvt. Ltd., Bombay, India; pyridoxine (pyridoxine monohydrochloride) and benzyl adenine (BA; 6 - benzyl amino purine N<sup>6</sup> benzyl adenine) from SIGMA, India, through commercial channels.

Series of experiments on broomrape germination were done by following various methods during August, 1992 to July, 1995 intermittently. The protocols in the various methods are as follows:

#### 1. Direct method

- a. wash Petri dishes thoroughly with tap water;
- b. keep the washed Petri dishes in the oven for sterilization for 24 hours;
- c. after sterilization, take out the Petri dishes from the oven and place 4.5 cm diameter filter paper (Whatman No. 42) on the bottom of the lower lid of the Petri dish and add distilled water on the filter paper just to moisten it, and then keep it in the oven for 24 hours for complete sterilization;
- d. take out the Petri dishes from the oven and wet the 4.5 cm diameter filter paper and put one tip pin of *Orobanche cernua* seeds (approximately 500 seeds) on the wetted filter paper and add 6 ml of a solution of known concentration of one of the chemicals gently without disturbing the seeds placed on the filter paper;
- e. keep the Petri dishes in an incubator at 25 °C and keep on adding 6 ml solution of various chemicals every day and observe for germination after 20 days of incubation under a stereo microscope;
- f. germination in obligate parasites is usually cryptocotylar, meaning that the cotyledons remain within the seed coat (Musselman and Press, 1995). Therefore, protruberance of a whitish radicle through the micropylar end of the seed is the

correct diagnosis of germination.

This method was carried out for four series, with three replicates each. These series are called Experiment 1 and the results (average over four series) are presented in Table 1.

## 2. Pre-soaking method

In this method, the seeds of *Orobanche cernua* were gently rubbed with zero sand paper and subsequently soaked in water. The pre-conditioning period was 10 days. Thereafter, the protocol already presented under "Direct method" was followed.

This method was also carried out for four series, with three replicates each. These series are called Experiment 2 and the average results are presented in Table 1.

## 3. Coir method

- a. follow steps a - c of the direct method but use blotting paper discs instead of filter paper discs;
- b. place about 500 *Orobanche cernua* seeds on a wet blotting paper disc (4.5 cm diameter) and cover with a similar disc;
- c. place the discs in a Petri dish and place "coir", i.e. coconut (*Cocos nucifera*) fibre pieces (wetted with water) on discs to a thickness of one centimeter;
- d. sow 10 crop seeds over the coir pad;
- e-1. water the bioassay unit daily, keep it in daylight for 30 minutes and place it back in the incubator at 25 °C; incubation should last 10 days; an alternative is to keep the bioassay unit under glasshouse conditions (see e-2);
- e-2. water the bioassay unit daily and keep it under the glasshouse conditions, placed under partial shade, where the temperature ranges from 27 - 30 °C; the greenhouse period should last 14 days;
- f. remove seedlings and fibre pieces pad and deblot the two wet blotting paper discs with dry blotting paper to remove the excessive water;
- g. examine the discs under a stereo microscope for assessing *Orobanche cernua* seeds germination;
- h. protruberance of a whitish radicle through the micropylar end of the seed is the correct diagnosis of the germinated seed.

This method was carried out for Experiments 3 - 7 (see below). It is similar to the *in situ* root absorption method described by Krishnamurthy et al. (1981) and the rapid bioassay test described by Krishnamurthy and Nagarajan (1991).

In Experiments 1 and 2, the treatments consisted of: GR24 at 0.1 and 1.0 ppm, GA at 10 and 20 ppm, ethephon at 2, 20 and 50 ppm, NAA at 1 and 20 ppm,

pyridoxine (vitamin B-6) at 1 and 10 ppm, BA at 0.2 and 2 ppm and a control (distilled water). These chemicals were selected on the basis of their possible effects reported in the literature (see introduction). GR24, a strigol analogue, was added as a standard to assess potential germination.

In Experiment 3, various crop seeds viz. sunhemp (*Crotalaria juncea*), greengram (*Vigna radiata*), redgram (*Cajanus cajan*), blackgram (*Vigna mungo*), pea (*Pisum sativum*), sunflower (*Helianthus annuus*), soybean (*Glycine max*), sesamum (*Sesamum indicum*) and control (only distilled water) were the treatments to induce the germination of *Orobanche cernua* seeds using the coir method both in the incubator and under glasshouse conditions. Similar trap crops were studied under field conditions (Dhanapal and Struik, 1996). This experiment was run for three series with three replications each for both incubator and glasshouse conditions and the results (average of three series) are presented in Table 2.

In Experiments 4 and 5, the coir method was used to test the effects of the same chemicals in the same concentrations for Experiments 1 and 2 with and without greengram seeds. Twenty nine treatment combinations were included: GR24 with and without greengram seeds at 0.1 and 1 ppm, GA with and without greengram seeds at 10 and 20 ppm, ethephon with and without greengram seeds at 2, 20 and 50 ppm, NAA with and without greengram seeds at 1 and 20 ppm, pyridoxine with and without greengram seeds at 1 and 10 ppm, BA with and without greengram seeds at 0.2 and 2 ppm, greengram seeds with distilled water, coir without greengram seeds and control (only distilled water). Experiment 4 was kept in the incubator, whereas Experiment 5 was put under glasshouse conditions. Experiment 4 included two series with three replications each and Experiment 5 included three series with three replications each (Table 3).

In Experiment 6, the combined effects of the root exudates of the same crop species as for Experiment 3 and GR24 at different concentrations on the germination were studied under glasshouse conditions (Table 4). There were 27 treatments: sunhemp, greengram, redgram, blackgram, pea, sunflower, soybean and sesamum combined with 0, 0.1 and 1.0 ppm GR24, and GR24 at 0, 0.1 and 1.0 ppm without crop seeds.

In Experiment 7, the effects of different cultivars of five crops with and without GR24 on the germination of the parasitic seeds were studied (Table 5). There were 33 treatments with greengram, blackgram, redgram, sunflower and soybean with two cultivars of each crop in combination with and without GR24 at 0, 0.1 and 1.0 ppm, GR24 at 0, 0.1 and 1.0 ppm without crop seeds.

#### Statistical procedures

Results are expressed in percentage germination.

There were no differences between series within one experiment. Both the

trends and the magnitudes of the effects were very consistent. Data were therefore pooled.

Germination percentages were arcsine-root transformed. Transformed data were subjected to a one-way ANOVA but the results of the original values are presented in the tables. Fischer's method of 'Analysis of Variance' was applied for the analysis and interpretation of the data. The level of significance used in 'F' and protected 't' tests was  $P=0.05$ . The values of 'F' and 't' and critical differences (LSD) were calculated following the method outlined for Completely Randomised Block Design by Panse and Sukhatme (1967).

### 4.3 Results

Table 1 reveals that the seed germination of the parasite was significantly affected by chemicals both when using the direct or the pre-soaking method of testing. Germination percentage of *Orobanche cernua* seeds was highest for GR24 at 0.1 and 1.0 ppm in both experiments, followed by GA at 10 or 20 ppm, and ethephon at 20 or 50 ppm. However, NAA, pyridoxine and BA had only a small, but significant effect on the germination percentage and these effects were mostly affected by the concentration used. Virtually no germination of broomrape seeds was observed in the control treatment. Conditioning of *Orobanche cernua* seeds with distilled water for a period of 10 days (pre-soaking method) generally enhanced germination (Table 1).

Germination was strongly stimulated by the root exudates of various crops (Table 2). Significant differences among the crops in their ability to induce germination of the parasite were observed both under incubator and glasshouse conditions. Maximum percentage of germination of the parasite was observed when the seeds were exposed to greengram, sunhemp and sesamum crops followed by blackgram and sunflower crops both in incubator and glasshouse conditions. Comparatively low percentages of germination of the parasite were found when induction had to be performed by redgram, soybean or pea. Germination in the control was negligible. Germination of the parasitic seeds was better in the glasshouse than in the incubator.

The effects of chemicals on the germination of *Orobanche cernua* seeds in the coir method are illustrated in Table 3. GR24 at 0.1 and 1.0 ppm concentrations significantly induced the germination of the parasite both under incubator and glasshouse conditions (Table 3). GR24 at 1.0 ppm was capable of inducing 34 - 45% of the seeds to germinate in the absence of a host. This was followed by GA at 10 and 20 ppm and ethephon at 20 ppm concentration. Significantly lower percentages of germination of the parasite were observed for the other chemicals tested, although the effects were much larger than in Experiments 1 and

2. When a host was present the germination was even more enhanced, especially in those treatments in which germination was already considerable without greengram seeds. The positive effect was much lower for the other treatments. Therefore, we could see clearly the interaction between the effect of greengram and the effect of the chemicals in stimulating the germination of the parasitic seeds. There was still a significant effect of the chemical when there was a host present. Virtually no germination could be seen in the control and in the treatment with coir and distilled water.

Table 1. Effect of chemicals on the germination of *Orobanche cernua* Loeffl. seeds by direct and pre-soaking methods under incubator conditions.

Chemicals	Concentration	Germination (%)	
		Direct method*	Pre-soaking method**
GR24	0.1 ppm	7.7 <sup>g</sup>	8.4 <sup>g</sup>
	1.0 ppm	8.9 <sup>g</sup>	8.7 <sup>g</sup>
Gibberellic acid	10 ppm	5.4 <sup>f</sup>	5.5 <sup>ef</sup>
	20 ppm	5.1 <sup>f</sup>	6.4 <sup>f</sup>
Ethephon	2 ppm	0.9 <sup>bc</sup>	2.3 <sup>b</sup>
	20 ppm	3.2 <sup>e</sup>	4.4 <sup>de</sup>
	50 ppm	3.1 <sup>e</sup>	3.8 <sup>d</sup>
Naphthalene acetic acid	1 ppm	1.0 <sup>bc</sup>	2.5 <sup>b</sup>
	20 ppm	2.4 <sup>de</sup>	3.6 <sup>cd</sup>
Pyridoxine	1 ppm	0.6 <sup>b</sup>	2.3 <sup>b</sup>
	10 ppm	1.6 <sup>cd</sup>	4.0 <sup>d</sup>
Benzyl adenine	0.2 ppm	0.5 <sup>b</sup>	2.8 <sup>bc</sup>
	2.0 ppm	2.0 <sup>de</sup>	2.7 <sup>bc</sup>
Control (distilled water)		0.0 <sup>a</sup>	0.4 <sup>a</sup>
P		<0.01	<0.01
CV (%)		12.7	11.6

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for transformed data

\* Experiment 1: Average of four series with three replications each (approx. 6000 seeds per treatment in total)

\*\* Experiment 2: Average of four series with three replications each (approx. 6000 seeds per treatment in total)

Table 2. Effect of various crops on the percentage germination of *Orobanche cernua* Loefl. seeds by the coir method under incubator and glasshouse conditions.

Trap crops	Germination (%)	
	Incubator*	Glasshouse*
Sunhemp ( <i>Crotalaria juncea</i> )	19.2 <sup>cd</sup>	24.4 <sup>d</sup>
Greengram ( <i>Vigna radiata</i> )	22.6 <sup>d</sup>	27.1 <sup>d</sup>
Redgram ( <i>Cajanus cajan</i> )	4.0 <sup>b</sup>	4.9 <sup>b</sup>
Blackgram ( <i>Vigna mungo</i> )	16.5 <sup>c</sup>	19.5 <sup>c</sup>
Pea ( <i>Pisum sativum</i> )	3.8 <sup>b</sup>	5.2 <sup>b</sup>
Sunflower ( <i>Helianthus annuus</i> )	14.8 <sup>c</sup>	16.6 <sup>c</sup>
Sesamum ( <i>Sesamum indicum</i> )	17.8 <sup>cd</sup>	23.6 <sup>d</sup>
Soybean ( <i>Glycine max</i> )	3.6 <sup>b</sup>	6.4 <sup>b</sup>
Control (distilled water)	0.2 <sup>a</sup>	0.8 <sup>a</sup>
	P < 0.01	< 0.01
	CV (%) 13.3	7.4

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data

\* Experiment 3: Average of three series, each with three replications (approx. 4500 seeds per treatment in total), for both incubator and glasshouse conditions



Table 3. Effect of chemicals on the percentage germination of *Orobancha cernua* Loefl. seeds by the coir method under incubator and glasshouse conditions.

Chemicals	Concentration	Germination (%)			
		Incubator*		Glasshouse**	
		No GG <sup>#</sup>	+ GG <sup>#</sup>	No GG <sup>#</sup>	+ GG <sup>#</sup>
GR24	0.1 ppm	27.2 <sup>e</sup>	41.8 <sup>g</sup>	42.8 <sup>kl</sup>	53.9 <sup>n</sup>
	1.0 ppm	33.5 <sup>f</sup>	44.5 <sup>g</sup>	45.2 <sup>lm</sup>	58.0 <sup>n</sup>
Gibberellic acid	10 ppm	25.7 <sup>de</sup>	32.5 <sup>f</sup>	33.7 <sup>jl</sup>	47.8 <sup>m</sup>
	20 ppm	23.0 <sup>bcd</sup>	31.2 <sup>ef</sup>	35.7 <sup>l</sup>	40.5 <sup>k</sup>
Ethephon	2 ppm	21.8 <sup>bcd</sup>	27.2 <sup>ef</sup>	21.0 <sup>cd</sup>	28.9 <sup>gh</sup>
	20 ppm	25.5 <sup>cde</sup>	32.5 <sup>f</sup>	26.0 <sup>efg</sup>	32.4 <sup>hij</sup>
	50 ppm	21.0 <sup>bcd</sup>	23.2 <sup>bcd</sup>	22.0 <sup>bcd</sup>	23.4 <sup>cde</sup>
Naphthalene acetic acid	1 ppm	20.3 <sup>bc</sup>	28.3 <sup>ef</sup>	23.2 <sup>bcd</sup>	30.4 <sup>hi</sup>
	20 ppm	19.0 <sup>b</sup>	24.5 <sup>bcd</sup>	19.9 <sup>bc</sup>	28.6 <sup>gh</sup>
Pyridoxine	1 ppm	21.2 <sup>bcd</sup>	25.7 <sup>bcd</sup>	22.3 <sup>bcd</sup>	25.0 <sup>def</sup>
	10 ppm	21.8 <sup>bcd</sup>	24.7 <sup>bcd</sup>	19.9 <sup>bc</sup>	23.0 <sup>bcd</sup>
Benzyl adenine	0.2 ppm	19.3 <sup>b</sup>	24.0 <sup>bcd</sup>	19.5 <sup>b</sup>	25.3 <sup>efg</sup>
	2.0 ppm	19.2 <sup>b</sup>	23.8 <sup>bcd</sup>	19.6 <sup>bc</sup>	24.3 <sup>def</sup>
Greengram + distilled water		-	23.5 <sup>bcd</sup>	-	21.4 <sup>bcd</sup>
Coir + distilled water		0.3 <sup>a</sup>	-	0.0 <sup>a</sup>	-
Control (distilled water)		0.0 <sup>a</sup>	-	0.0 <sup>a</sup>	-
	P	<0.01		<0.01	
	CV (%)	7.4		5.3	

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data

\* Experiment 4: Average of two series with three replications each (approx. 3000 seeds per treatment in total)

\*\* Experiment 5: Average of three series with three replications each (approx. 4500 seeds per treatment in total)

# Greengram seeds

Table 4 shows the interaction between GR24 and different trap crops. Significantly higher levels of germination of broomrape were obtained when greengram, sunhemp, blackgram, sesamum and sunflower were present as compared to treatments with redgram, pea or soybean in the absence of GR24. GR24 at 0.1 ppm strongly enhanced germination but did not allow the positive effect of the trap crop to express itself significantly. GR24 at 1.0 ppm stimulated the germination even more than GR24 at 0.1 ppm and with the highest concentration positive effects of the germinating trap crops were again observed. This suggests that natural stimulants may even have an effect on germination when there is already a strong induction by GR24.

Table 4. Effect of trap crop with and without GR24 on the percentage germination of *Orobancha cernua* Loefl. seeds by the coir method under glasshouse conditions. Experiment 6: One series with three replications (approx. 1500 seeds per treatment in total).

Trap crop	GR24 (parts per million)		
	0	0.1	1.0
Sunhemp	20.3 <sup>c</sup>	47.7 <sup>defg</sup>	57.7 <sup>i</sup>
Greengram	22.0 <sup>c</sup>	48.0 <sup>defg</sup>	56.7 <sup>j</sup>
Redgram	5.3 <sup>b</sup>	46.7 <sup>def</sup>	54.3 <sup>ghi</sup>
Blackgram	17.7 <sup>c</sup>	46.7 <sup>def</sup>	58.7 <sup>j</sup>
Pea	6.7 <sup>b</sup>	45.3 <sup>de</sup>	51.3 <sup>efghi</sup>
Sunflower	18.3 <sup>c</sup>	46.7 <sup>def</sup>	51.7 <sup>ghi</sup>
Sesamum	19.3 <sup>c</sup>	47.3 <sup>defg</sup>	55.7 <sup>hi</sup>
Soybean	5.3 <sup>b</sup>	44.3 <sup>de</sup>	54.0 <sup>fghi</sup>
Range	5.3-22.0	44.3-48.0	51.3-58.7
No crop	0.0 <sup>a</sup>	41.3 <sup>d</sup>	48.3 <sup>defgh</sup>
	P	<0.01	
	CV (%)	7.68	

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data.

Table 5. Effect of cultivars of trap crops with and without GR24 on the percentage germination of *Orobancha cernua* Loefl. seeds by the coir method under glasshouse conditions. Experiment 7: One series with three replications (approx. 1500 seeds per treatment in total).

Trap crop	Cultivar	GR24 (parts per million)		
		0	0.1	1.0
Greengram	PS-16	23.0 <sup>de</sup>	49.3 <sup>ghij</sup>	58.7 <sup>k</sup>
	KGG-1	23.3 <sup>e</sup>	48.7 <sup>ghij</sup>	59.3 <sup>k</sup>
Blackgram	T-9	18.0 <sup>cde</sup>	46.0 <sup>fg</sup>	59.3 <sup>k</sup>
	K-3	16.7 <sup>cd</sup>	47.0 <sup>gh</sup>	58.7 <sup>k</sup>
Redgram	TTB-7	6.7 <sup>b</sup>	47.3 <sup>fghi</sup>	55.3 <sup>jk</sup>
	Hyd-3C	5.7 <sup>b</sup>	47.3 <sup>fghi</sup>	55.0 <sup>ijk</sup>
Soybean	Hardee	6.7 <sup>b</sup>	44.0 <sup>fg</sup>	54.3 <sup>hijk</sup>
	KHSB	3.7 <sup>b</sup>	46.7 <sup>fgh</sup>	49.7 <sup>ghij</sup>
Sunflower	Morden	15.0 <sup>c</sup>	48.0 <sup>fghi</sup>	55.0 <sup>ijk</sup>
	KBSH-1	16.7 <sup>cd</sup>	46.0 <sup>fg</sup>	54.0 <sup>hijk</sup>
Range		3.7-23.3	44.0-49.3	49.7-59.3
No crop		0.0 <sup>a</sup>	40.7 <sup>f</sup>	49.3 <sup>ghij</sup>
	P		<0.01	
	CV (%)		7.69	

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data.

The effect of cultivars of different trap crops with and without GR24 on the germination of *Orobancha cernua* seeds by the coir method under glasshouse conditions is presented in Table 5. The overall results are very similar to the ones of the previous experiment: GR24 enhanced germination even in the presence of a host, whereas there were significant differences in efficiency of hosts, especially at 0 and 1 ppm GR24. The host effect was still significant, even when GR24 was

applied at high concentrations. The difference in stimulating effect on the germination of the parasite among cultivars of the same crop was never significant.

#### 4.4 Discussion

Broomrape seeds are most vulnerable to destruction immediately after germination but prior to the attachment of the germ tube to host roots. If broomrape seeds are induced to germinate in the absence of host plants, the seedlings are unable to support themselves and soon die.

The objectives of this study were to develop a suitable method by standardising the technology with which one could induce the germination of *Orobancha cernua*, identifying certain promising chemicals with their optimal concentrations under laboratory conditions and to test the ability of trap crops with their cultivars either in the absence or presence of chemicals to enhance the germination of *Orobancha cernua* seeds. Later, these chemicals can be tried with or without trap crops under field conditions.

The results of the present investigation showed very little variation among different series but large variations from one method to another, both under incubator and glasshouse conditions, which is often found in research on the seed germination of broomrapes. Basler (1981) reported that the germination of seed of the same origin can vary to a large extent and over a short period. He suggested that the variation is due to dormancy which is controlled by endogenous factors.

In the present study, the low germination percentage of *Orobancha cernua* in Experiments 1 and 2 may be attributed to the reasons that the prolonged wetting might have induced secondary dormancy, lack of surface sterilization or pre-soaking of the seeds (except in the pre-soaking method) before treating the seeds with chemicals or natural stimulants. The 'coir method' proved an excellent bioassay method to induce the germination of *Orobancha cernua*.

The germination percentage of the parasite was higher in glasshouse conditions than under incubator conditions in Experiments 3 - 7. This may be due to the fact that the seedlings grown in glasshouse conditions under partial shade were sturdy and had green leaves. On the other hand, crop seedlings grown in the incubator were lanky and etiolated, and could only be maintained for 10 days.

GR24 at 0.1 and 1.0 ppm, GA at 10 and 20 ppm and ethephon at 20 ppm induced the germination of *Orobancha cernua* seeds better than the other chemicals, both in direct and pre-soaking methods. These effects may be related: germination stimulants may play a role in ethylene biosynthesis, thus enhancing germination (Boone et al., 1995). Relatively more germination was observed in the pre-soaking method. This may be due to the rubbing of *Orobancha cernua* seeds with zero sand paper. Moreover, the pre-soaking period might have broken the

dormancy and may have provided better conditions for imbibition.

It is evident from Experiment 3 that the *in situ* production and supply of root exudates from the seedlings of various crops induced the germination of the parasitic seeds. The results confirm field studies (Dhanapal and Struik, submitted): sunhemp and greengram are promising trap crops, whereas redgram, sunflower, and pea trap crops were found to be less effective in inducing the germination of *Orobanche cernua* seeds in a rotation of tobacco - (fallow) - trap crop - tobacco. High levels of germination were obtained after 10 days under incubator conditions and 14 days in glasshouse conditions, suggesting that root production after such a period was sufficient to provide the root exudates required for inducing the germination of *Orobanche cernua* seeds.

GR24, a strigol analogue, at 0.1 ppm and 1.0 ppm concentrations was extremely effective in inducing the germination of *Orobanche cernua* seeds, both in the incubator and glasshouse conditions in all the methods. GR24, GR28 and GR41 stimulated the germination of *Orobanche minor* seeds in the laboratory (Spelce and Musselman, 1981; Parker and Riches, 1993). They also reported that maximum germination of broomrape seeds was obtained with these strigol analogues at 0.1 to 1.0 ppm when seeds were pre-conditioned with water and incubated for 15 days at 25 °C. The isolation and purification of strigol in large amounts from root exudates appears impractical (Vail et al., 1985); an artificial synthesis of GR24 or related compounds is necessary to obtain enough compounds for extensive field testing or for commercial use (Zwanenburg et al., 1994). The use of strigol or its analogues as seed germination stimulants in the field has not yet been possible due to the high cost involved in their synthesis and also their instability in soils with high pH, which are common in broomrape-infested areas. Strigol and its analogues remain of primary interest as research tools due to their activity not only on parasitic seeds but also on a few autotrophic weeds. An insight into the mechanism of action of strigol and its analogues may help to develop chemicals that have similar activity and can be synthesized economically. The isolation and identification of the factor in host root exudates may also open new avenues into the chemistry of germination stimulants. The GR24 compound gave maximum germination of *Orobanche aegyptiaca*.

The combination of chemicals and trap crops showed an interaction in stimulating the germination. Partly additive effect of natural stimulants is more pronounced in the presence of GR24 at 1.0 ppm than at 0.1 ppm concentration. The additional effect of natural stimulants is partly hidden by GR24 at 0.1 ppm.

Gibberellic acid at 10 ppm and 20 ppm stimulated the germination of the parasitic seeds both in the incubator and under glasshouse conditions, by direct as well as the coir methods either with greengram or without greengram as a trap crop. This is consistent with literature (Al-Menoufi, 1986; Hiron, 1973). In our

study, GA performed better than the other chemicals but was considerably less effective than GR24 in stimulating the germination of *Orobancha cernua* seeds.

Reports on the effect of ethephon on broomrape seed germination are scarce. In our experiments, ethephon (a.i. ethylene) at 50 ppm reduced the germination of *Orobancha cernua* seeds in the coir method when compared to 2 ppm and 20 ppm suggesting that higher concentrations show signs of inhibition of *Orobancha cernua* seeds germination. Also Chun et al. (1979) observed that ethylene at high concentrations inhibited broomrape seed germination instead of stimulating it.

Auxins such as indole acetic acid and naphthalene acetic acid and cytokinins have been found to stimulate the germination of broomrape seeds to some extent (Nash and Wilhelm, 1960; Edwards et al., 1976; Strelyaeva, 1978). NAA, pyridoxine and BA, however, were found less effective in stimulating the germination of *Orobancha cernua* seeds.

We also observed interactions between natural host stimulant by growing greengram seedlings and different chemicals both in incubator and glasshouse conditions. An enhanced germination percentage of the parasite by greengram seedlings was observed in the chemical treatments when there was already a strong stimulation by the chemical themselves; especially greengram with GR24 at 0.1 and 1.0 ppm followed by GA at 10 and 20 ppm and ethephon at 2 and 20 ppm concentrations. This positive effect of greengram was not pronounced in the other chemicals tested because such chemicals induced low germination in the absence of greengram seedlings. Ethephon at 50 ppm with greengram also showed less germination indicating its toxicity at higher concentrations. Similar observations were made by Chang et al. (1979).

It can be concluded that the stimulatory effect of the different chemicals, trap crops or combination of chemicals and crops on the germination of *Orobancha cernua* seeds was different in different methods under incubator or glasshouse conditions. GR24 at 0.1 and 1.0 ppm was found extremely effective in stimulating the germination of the parasitic seeds followed by GA at 10 ppm and 20 ppm concentrations, especially under glasshouse conditions using the coir method. Even under such conditions there is an additional effect of trap crops.

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## Chapter 5

## Chapter 5

### STUDIES ON THE POST-EMERGENCE CONTROL OF BROOMRAPE IN TOBACCO WITH HERBICIDES

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#### Summary

The effectiveness of herbicides applied at different dates and in different concentrations in controlling *Orobanche cernua* and their effects on growth and yield of tobacco were evaluated at the Agricultural Research Station, Nipani, India during 1992-93 and 1994-95. Glyphosate and imazaquin were found promising in controlling the parasite without a large reduction in the tobacco yield. Glyphosate at 0.50 kg a.i./ha applied at 60 days after transplanting and imazaquin at 0.01 kg a.i./ha applied at 30 days after transplanting reduced the number of above-ground broomrape spikes by 75 - 80% and increased the tobacco yields by 43% as compared to the control treatment. Imazethapyr and EPTC were found less effective. The hand weeding treatment gave highest yields of the harvestable product. Treatment effects showed the same trends on the dry weight of leaves for both "infested" and "non-infested" tobacco plants.

Toxicity of all herbicides tested was higher for the higher concentrations within the range tested.

#### 5.1 Introduction

*Orobanche cernua* Loefl. is an obnoxious, debilitating holoparasitic weed in all tobacco growing areas in India, threatening the tobacco cultivation with its devastating effect on the crop. Various methods have been evaluated to control *Orobanche* in different crops including mechanical, cultural, biological, genetic and chemical ones (Pieterse, 1979; Saghir et al., 1980; Foy et al., 1989; Sauerborn et

al., 1989; Garcia-Torres et al., 1994) and indirect methods by using growth regulators which may cause some physiological changes in the host or the parasite (El-Ghamrawy and Neumann, 1991; Hassan et al., 1991). However, none of these methods proved to give consistent, effective or economic control of the parasite (Saghir et al., 1980; Jacobsohn, 1986; Lolas, 1986; Foy et al., 1989; Nemli et al., 1989; Kotoula-Syka and Elephtherohorinos, 1991; Parker, 1991; Joel, 1992; Garcia-Torres et al., 1994; Goldwasser et al., 1994; Kleifeld et al., 1994). Many chemicals have been tested for their effects on broomrape; recently some results were summarized by Eplee and Norris (1995). Such research is relevant: removal of broomrape by mechanical means is not always feasible because labour is scarce and expensive, and the parasite emerges close to the host plants; moreover, hand pulling of broomrape is not very effective in heavy infestations, after the crop has sustained damage and broomrapes can infest the host plant from a depth of 30 cm. The herbicide technology approach offers the possibility of the simplest, safest and most effective possible solution.

Selective control of *Orobanche* based on herbicide - plant interaction or differential response is very difficult due to the close ecological and physiological relationship of the parasite with its host plants (Anderson, 1977). Application of herbicides either to soil or foliage to control *Orobanche* after attachment to the host roots has reported to be effective or partially effective but also injurious to crop plants (Jacobsohn, 1986; Foy et al., 1989; Sauerborn et al., 1989; Garcia-Torres and López-Granados, 1991; Kotoula-Syka and Elephtherohorinos, 1991; Goldwasser et al., 1992; Joel, 1992; Khalaf et al., 1994).

Herbicides without an effect on the tobacco crop but effective in controlling *Orobanche cernua* (or at least its seed formation) are lacking in India. The objectives of this investigation were first to screen 15 herbicides in a primary screening trial to test their phytotoxic effects on the parasite and to test their selectivity in the bidi tobacco crop. In the secondary trials, the herbicides which performed best in the primary screening trial were further evaluated. The effects of the herbicides were analysed, both on tobacco plants "infested" and "non-infested" with *Orobanche cernua* (i.e. plants with and without emerged broomrape spikes) and on the parasite. Results were compared with the effects of hand weeding and a non-treated control.

## 5.2 Materials and Methods

### Site characteristics

Three separate field experiments were conducted at the Agricultural Research Station, Nipani, Karnataka State, India during 1992-93, 1993-94 and 1994-95. The research station is located at 16° 2' North latitude and 74° 2' East longitude, at an

altitude of 610 metres above sea level, on a black clay loam having 50 - 55% silt and 20 - 25% clay with a field capacity of 40%. The pH of the soil is 8.3. The area experiences dry hot summers, humid and cool monsoons and mild winters. The average rainfall is 720 mm (average over 40 years) and 60% of the total precipitation is received during June to August with an average of 82 rainy days per year. Relative humidity fluctuates between 38% and 85% (average of 14 years). Mean monthly maximum temperature ranges from 29.8 °C (December) to 37.5 °C (April) and mean monthly minimum temperature ranges from 13.6 °C (December) to 21.9 °C (May) (average of 14 years).

#### Experimental set-up

A bulk crop bidi tobacco was grown during 1991-92 as a preceding crop on the experimental plot and later the land was kept fallow for six months. A field experiment for primary screening was conducted during the 1992-93 late kharif (spring; rainy period) and rabi (winter) seasons. Crop management was as normal for the region. No irrigation was applied. The treatments consisted of 15 herbicides at different concentrations each at 30 and 60 days after transplanting tobacco (Table 1).

The experiment was laid out in a tobacco crop naturally infested with *Orobanche cernua* in a completely randomised block design. For each herbicide treatment, five tobacco plants were randomly selected and tagged with labels. One square metre area was marked around each labelled tobacco plant for spraying chemicals and all the observations were recorded in the 1.0 m<sup>2</sup> area.

During the 1993-94 late kharif (spring; rainy period) and rabi (winter) seasons, a second field experiment was conducted in a tobacco field naturally infested with *Orobanche cernua*. The experimental plot was kept fallow for nearly six months and prior to that bulk bidi tobacco was grown (during 1992-93). Four herbicides selective to tobacco and toxic to *Orobanche cernua* as found in the primary screening trial were tested. Treatments consisted of: **glyphosate** (Round up<sup>®</sup>) at 0.5 and 1.0 kg a.i./ha, **imazaquin** (Scepter<sup>®</sup>) at 0.01 and 0.02 kg a.i./ha, **imazethapyr** (Pursuit<sup>®</sup>) at 0.015 and 0.030 kg a.i./ha and **EPTC** (Eptam<sup>®</sup>) at 0.5 and 1.0 kg a.i./ha, each at 30 and 60 days after transplanting tobacco, a hand weeding treatment and a control (Table 1). These 18 treatments combination were laid out in a randomised block design with three replications. The plot size was 8 × 4.5 m<sup>2</sup> and all the biometric observations were recorded in a net plot of 6 × 3 m<sup>2</sup>. Heavy and continuous rains, 40 - 45 days after transplanting tobacco created waterlogged conditions for about 8 - 10 days. Waterlogging is deleterious to broomrape (Mohamed-Ahmed and Drennan, 1994) and tobacco is also sensitive to waterlogging. Therefore there was 100% damage to the crop. Henceforth, the experiment conducted during 1993-94 was vitiated without recording any

Table 1. Chemicals used in the primary screening and their concentration. Chemicals indicated with \* were also tested in the secondary trials.

Chemical name	Trivial name	Concentration
N-(phosphono methyl) glycine	glyphosate*	0.5 and 1.0 kg a.i./ha
N-(1-ethyl propyl)-2, 6-dinitro-3, 4-xylidine	pendimethalin	0.5 and 1.0 kg a.i./ha
5-tert-butyl-3-(2, 4-dichloro-5-isopropoxy phenyl)-3,4-oxadiazol-2 (3H)-one	oxadiazon	0.75 and 1.0 kg a.i./ha
4-chloro-o-tolyloxy acetic acid	MCPA	0.5 and 1.0 kg a.i./ha
butyl-2-(4-(5-trifluoro methyl-2-pyridoxy) phenoxy propionate	fluazifop-p-butyl	1.0 and 2.0 kg a.i./ha
N-(2-chloroethyl)-2,6-dinitro-N-propyl-4-(trifluoro methyl) aniline	fluchloralin	1.0 and 2.0 kg a.i./ha
2-chloro- $\alpha, \alpha, \alpha$ -trifluoro-p-tolyl 3-ethoxy-4-nitrophenyl ether	oxyfluorfen	0.2 and 0.3 kg a.i./ha
(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) quinoline-3-carboxylic acid	imazaquin*	0.01 and 0.02 kg a.i./ha
2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid	imazapyr	0.005 and 0.010 kg a.i./ha
(RS)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid	imazethapyr*	0.015 and 0.030 kg a.i./ha
3-(4-isopropyl phenyl)-1,1-dimethyl urea	isoproturon	0.5 and 1.0 kg a.i./ha
S-ethyl dipropyl thiocarbamate	EPTC*	0.5 and 1.0 kg a.i./ha
methyl 2-[ [ [ N-(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-methyl amino ] carbonyl ] amino ] sulfonyl ] benzoate	tribenuron methyl	0.015 and 0.030 kg a.i./ha
2-( ( [4-chloro-6-methoxy pyrimidine-2-yl] amino carbonyl] amino sulfonyl) benzoic acid ethyl ester	chlorimuron	0.015 and 0.030 kg a.i./ha
methyl 2-[ [ [ (4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) amino ] carbonyl ] amino ] sulfonyl ] benzoate	metsulfuron methyl	0.015 and 0.030 kg a.i./ha
Control	-	-

observations. After a lapse of 10 months (land was kept fallow) during the 1994-95 late kharif and rabi seasons, a similar experiment was carried out in the same way as mentioned above.

### Cultural practice

In the primary screening trial, after ascertaining the fine tilth of the soil and sufficient moisture, healthy tobacco seedlings (variety: Anand-119) raised under optimal conditions were transplanted on 01.08.1992 at an inter-row spacing of 1.0 m and an intra-row spacing of 0.75 m. Thus, the plant density was 1.33/m<sup>2</sup>. Fertilizers at the rate of 125 kg N/ha in the form of ammonium sulphate, 27.5 kg P/ha in the form of single super phosphate and 31.1 kg K/ha in the form of sulphate of potash were applied before transplanting. At appropriate times, suitable cultural and plant protection measures were undertaken against tobacco pests and diseases other than *Orobanche*.

In the third trial tobacco seedlings (variety: Anand-119) were transplanted on 05.08.1994 by opening furrows with the help of a pair of bullocks at a distance of 1.0 m apart and a within row distance of 0.75 m. The plant density was 1.33/m<sup>2</sup>. The fertilizer application, cultural and plant protection measures were undertaken in the same way as mentioned for the primary screening trial. Herbicides in different concentrations were sprayed at 30 and 60 days after transplanting (DAT) tobacco by means of a backpack sprayer using 0.06 litres of water per m<sup>2</sup>. The tobacco crop took 185 days to complete maturity in both experiments described, then it was harvested. The leaves were harvested in the morning hours and kept in the respective plots for air-curing for 6 days.

### Observations

During 1992-93, in the primary screening trial, crop toxicity and weed control ratings (at 1, 3 and 5 days after spraying), number of broomrape spikes, broomrape dry weight, plant height of tobacco, dry weight and cured leaf yield of tobacco at harvest were recorded. Based on these observations, herbicides, viz. glyphosate, imazaquin, imazethapyr and EPTC found safe to tobacco and efficient in controlling *Orobanche cernua*, were selected for further study. All other herbicides were either partially effective or too injurious to tobacco and were not tested further.

During 1994-95, in the third trial, biometric observations were recorded both for the parasite and the host plant. The spike density of *Orobanche cernua* was counted on 60, 75, 90, 105 and 120 days after transplanting and at final harvest (185 days after transplanting) in the net plot area of 18 m<sup>2</sup>. Also, the spikes of *Orobanche cernua* were collected from a random area of 1.0 m<sup>2</sup> from each treatment within the net plot area on 60, 75, 90, 105 and 120 days after transplanting and at harvest. These spikes were air dried and then oven dried at 70

°C and the dry weights were recorded. At harvest, all *Orobanche cernua* spikes present in the remaining net plot area were pulled out and dried in the sun for a week and their dry weight was recorded.

The growth and yield of tobacco were recorded at different growth stages. Average plant height was recorded from five randomly selected plants on 45, 60, 90 and 120 DAT and at final harvest. Fresh and dry leaf weight (g/plant), dry weight of stem (g/plant) and total dry matter (g/plant) were assessed from two randomly selected "infested" and "non-infested" tobacco plants. The term "infested" refers to tobacco plants on which *Orobanche cernua* spikes were seen above-ground, whereas in "non-infested" plants *Orobanche cernua* spikes were not seen above-ground although they might be infected below-ground. Drying of the leaves and stems was done by air drying followed by oven drying at 60 - 65 °C at 60, 90 and 120 days after transplanting and at harvest. The weed (*Orobanche*) index, which is the reduction in crop yield due to the presence of weeds (*Orobanche*) in comparison with the crop yield obtained in a weed (*Orobanche*) free check (Gill and Vijayakumar, 1969) was calculated by the following formula:

$$W = \frac{X - Y}{X} \times 100$$

where

W = weed (*Orobanche*) index for crop Y

X = crop yield from weed (*Orobanche*) free check plot (kg/ha)

Y = crop yield from the treatment for which the weed index has to be calculated (kg/ha)

Above ground crop yield and leaf dry weight were recorded for each plot and expressed in g/m<sup>2</sup>. The curing of leaves was done through air-curing and the cured leaf yield in bidi tobacco constitutes four leaf components, viz. the lamina portion, midribs, sand leaves, and veins and veinlets.

#### Statistical procedures

The data on broomrape spike density and their dry weights at different timings were square root transformed to obtain homogeneity of variance. Fischer's method of 'Analysis of Variance' was applied for the interpretation of the data. The level of significance used in 'F' and 't' tests was P=0.05. The values of 'F' and 't' and least significant differences (LSD) were calculated following the method outlined for a Completely Randomised Block Design (primary screening trial) and a Randomised Block Design (second trial) by Panse and Sukhatme (1967).



### 5.3 Results

In the primary screening trial, based on the crop toxicity and weed control ratings, broomrape spike density, broomrape spike dry weight, plant height of tobacco, dry weight and cured leaf yield of tobacco recorded at harvest during 1992-93, glyphosate, imazaquin, imazethapyr and EPTC were found to be safe to tobacco and efficient in controlling *Orobanche cernua* and they were selected for further testing in the second and third trial. Pendimethalin and fluazifop-p-butyl at their higher concentrations and all other herbicides were either partially effective or too injurious to the tobacco crop and were not tested further (Table 2).

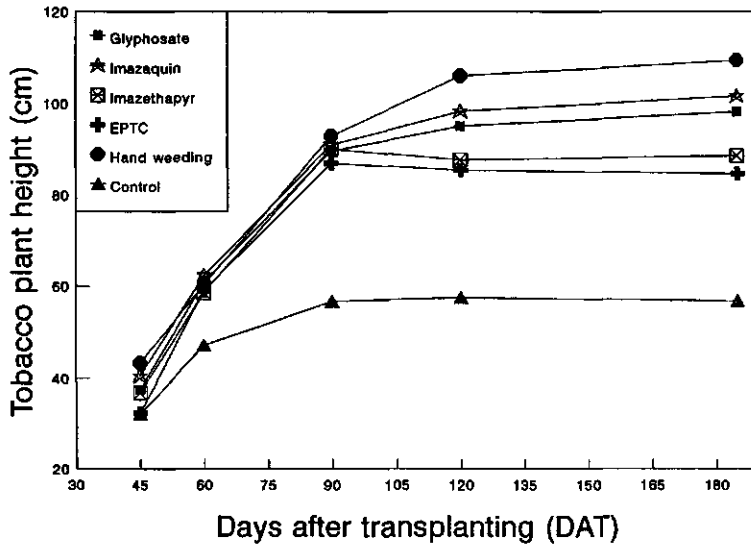
Table 2. Selective herbicides for tobacco found in the primary screening trial during 1992-93.

Chemical	Dose 1	Dose 2
Glyphosate	+	+
Pendimethalin	+ *	-
Fluazifop-p-butyl	+ *	-
Imazaquin	+	+
Imazethapyr	+	+
EPTC	+	+

\* toxic to host plant in the early growth stages but host plants recovered later.

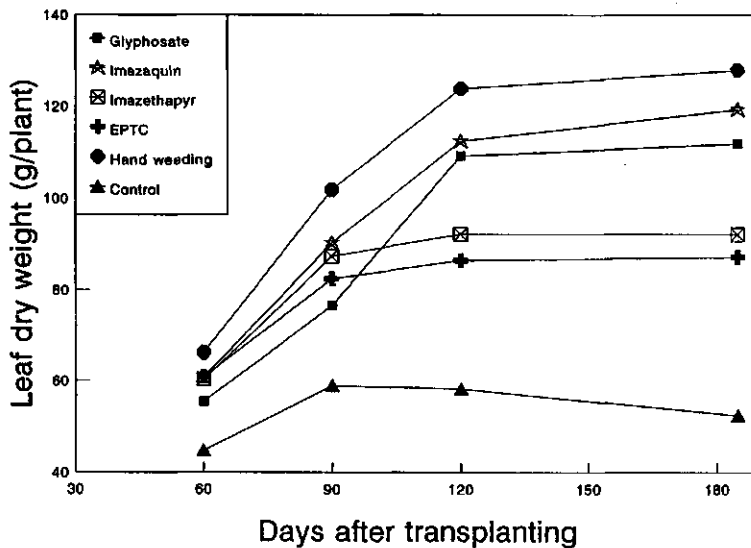
In Figures 1 - 4, effects of the best concentrations of the earlier application of the chemicals on the parasite and the growth and yield parameters of the host plants were compared to the hand weeding and control treatments. Plant height of tobacco from 45 - 90 days after transplanting was not reduced by the different herbicides compared to the hand weeding treatment; thereafter, plant height for treatments EPTC at 0.50 kg a.i./ha and imazethapyr at 0.015 kg a.i./ha applied at 30 days after transplanting did not increase any further, in contrast to the plant height in treatments imazaquin (30 DAT), glyphosate (30 DAT) and hand weeding (Fig. 1). Plant height was strongly reduced in the control treatment by the broomrape infection; maximum plant height was already reached at 90 DAT.

Dry weight of leaves (the harvestable product) of "infested" tobacco plants was highest in the hand weeding treatment followed by imazaquin and glyphosate (Fig. 2). Glyphosate initially impeded the increase in leaf dry weight, but plants recovered later. The final dry weight of leaves was lower in imazethapyr and EPTC



Glyphosate @ 0.5 kg a.i./ha at 30 DAT; Imazaquin @ 0.01 kg a.i./ha at 30 DAT  
 Imazethapyr @ 0.015 kg a.i./ha at 30 DAT; EPTC @ 0.5 kg a.i./ha at 30 DAT; Harvest: 185 DAT  
 LSD (0.05): 45 DAT: 4.9; 60 DAT: 3.1; 90 DAT: 4.8; 120 DAT: 3.9; Harvest: 4.1

Fig. 1. Effect of herbicides on the plant height of tobacco over time.



Glyphosate @ 0.5 kg a.i./ha at 30 DAT; Imazaquin @ 0.01 kg a.i./ha at 30 DAT  
 Imazethapyr @ 0.015 kg a.i./ha at 30 DAT; EPTC @ 0.5 kg a.i./ha at 30 DAT; Harvest: 185 DAT  
 LSD (0.05): 60 DAT: 9.0; 90 DAT: 7.4; 120 DAT: 7.9; Harvest: 8.1; DAT: Days after transplanting

Fig. 2. Effect of herbicides on the leaf dry weight of infested tobacco plants over time.

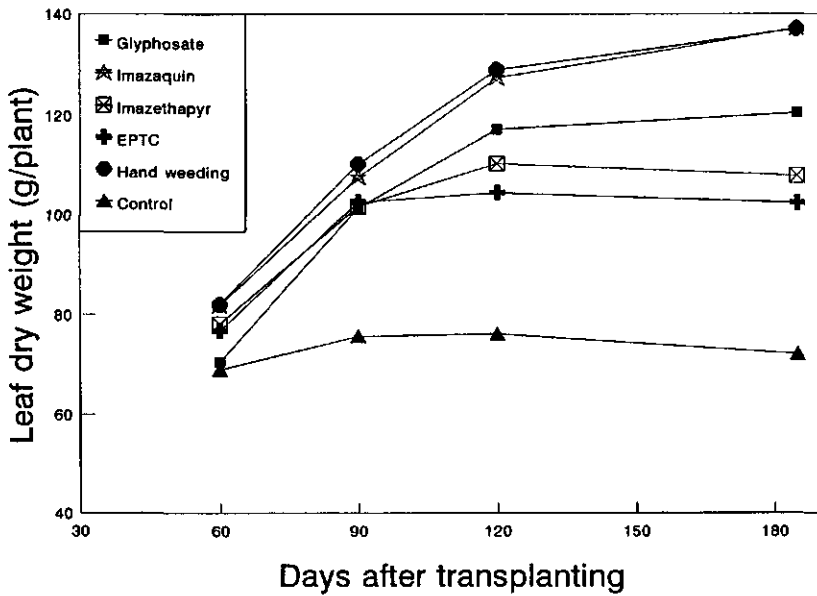
treatments than in the other herbicides and the hand weeding treatment, but significantly higher than in the control. Lowest dry weight of leaves was obtained in the control treatment. The trends in dry weights of leaves of "infested" and "non-infested" tobacco plants were similar (Fig. 3), but values were much higher in "non-infested" tobacco plants and the differences between the hand weeding, imazaquin and glyphosate were smaller.

The biological yield of tobacco (the above-ground crop yield) averaged over "infested" and "non-infested" tobacco plants as influenced by herbicides over time is depicted in Figure 4. Highest biological yield of tobacco was obtained in the hand weeding treatment followed by imazaquin and glyphosate. Significantly lower biological yield of tobacco was obtained in the control. Imazethapyr and EPTC were intermediate.

The effects of all treatments on plant height and yields at final harvest are given in Figures 5 - 8. Higher concentrations of all herbicides tested reduced plant height of tobacco as compared to the hand weeding and lower concentrations of the herbicides (Fig. 5). Imazaquin, imazethapyr and EPTC applied at 30 DAT and glyphosate at 60 DAT at their lower concentrations gave taller plants than at the other dates. No chemical treatment was better than the hand weeding or worse than the control.

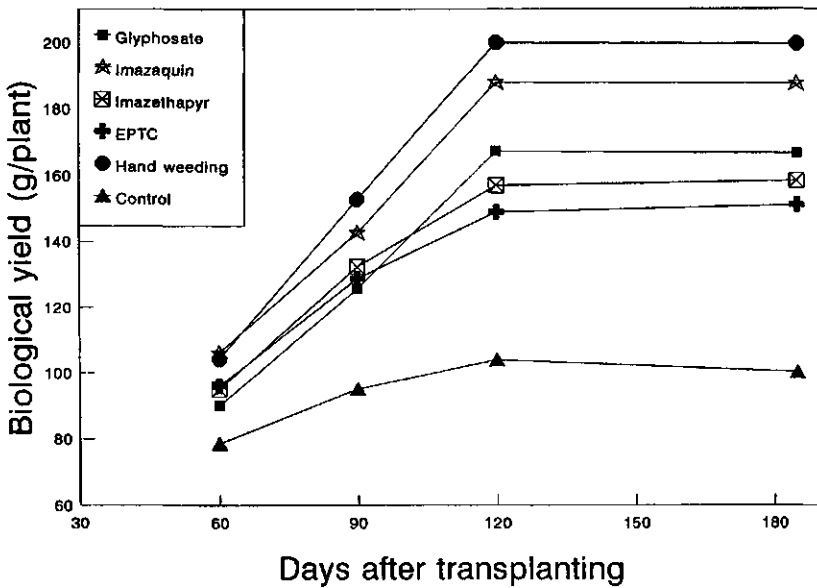
Highest dry weight of leaves from the "infested" tobacco plants were obtained at harvest in the hand weeding treatment, whereas significantly lowest tobacco leaf dry weight was recorded in the untreated tobacco plots (Fig. 6). Again, higher concentrations of herbicides reduced the dry weight of tobacco leaves significantly compared to their lower concentrations and the hand weeding treatment. Higher biological yields of tobacco were obtained when imazaquin, imazethapyr or EPTC were applied at 30 DAT than at 60 DAT; better time of application of glyphosate was 60 DAT. Similar trends were observed in the "non-infested" tobacco plants although yields were higher (Fig. 7) and for biological yields of tobacco at harvest (Fig. 8).

High numbers and dry weights of broomrapes were found in the control treatment (Figs 9 and 10). In the hand weeding treatment, virtually no broomrapes were seen because they were weeded out as and when they emerged above-ground. Significant reduction in broomrape spike densities was obtained in all herbicide treatments. Differences among herbicides were not large. Significantly highest dry weight of broomrape was obtained in the control treatment over a period of time (Fig. 10). Lower dry weights of broomrapes were recorded in the herbicide treatments. Especially glyphosate at 1.0 kg a.i./ha applied at 60 DAT and imazaquin at 0.02 kg a.i./ha applied at 30 days DAT reduced the broomrape spike density and yield significantly compared to imazethapyr at 0.03 kg a.i./ha and EPTC at 1.0 kg a.i./ha applied at 30 days after transplanting tobacco.



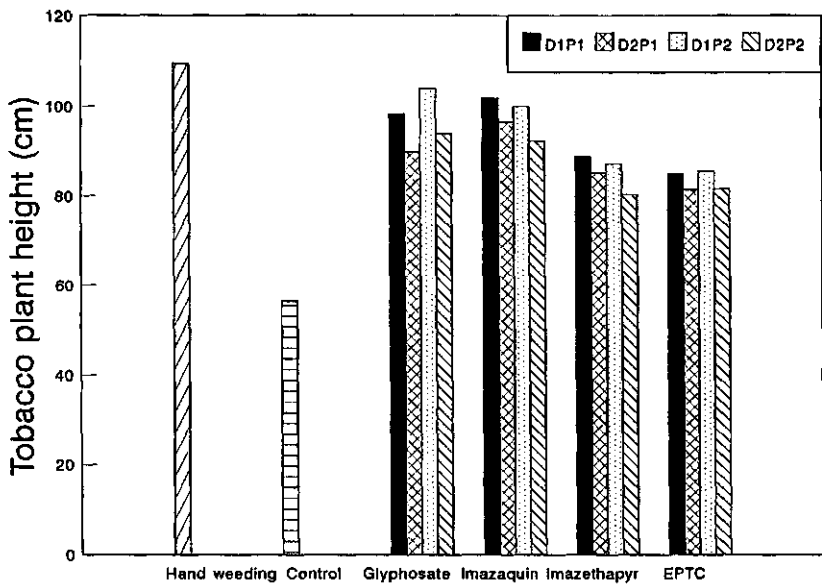
Glyphosate @ 0.5 kg a.i./ha at 30 DAT; Imazaquin @ 0.01 kg a.i./ha at 30 DAT  
 Imazethapyr @ 0.015 kg a.i./ha at 30 DAT; EPTC @ 0.5 kg a.i./ha at 30 DAT; Harvest: 185 DAT  
 LSD (0.05): 60 DAT: NS; 90 DAT: 10.7; 120 DAT: 8.0; Harvest: 6.6; DAT: Days after transplanting

Fig. 3. Effect of herbicides on the leaf dry weight of non-infested tobacco plants over time.



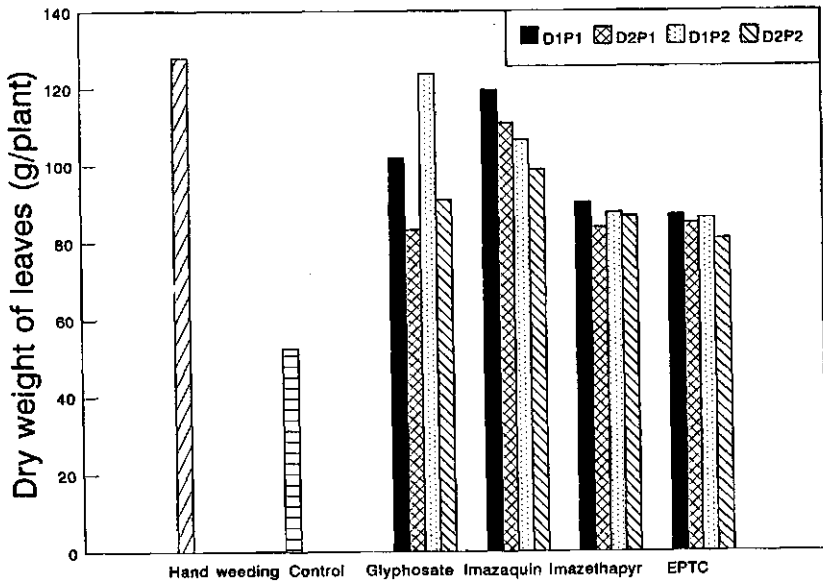
Glyphosate @ 0.5 kg a.i./ha at 30 DAT; Imazaquin @ 0.01 kg a.i./ha at 30 DAT  
 Imazethapyr @ 0.015 kg a.i./ha at 30 DAT; EPTC @ 0.5 kg a.i./ha at 30 DAT; Harvest: 185 DAT  
 LSD (0.05): 60 DAT: 9.0; 90 DAT: 10.0; 120 DAT: 9.3; Harvest: 10.2; DAT: Days after transplanting

Fig. 4. Effect of herbicides on the biological yield of tobacco plants over time.



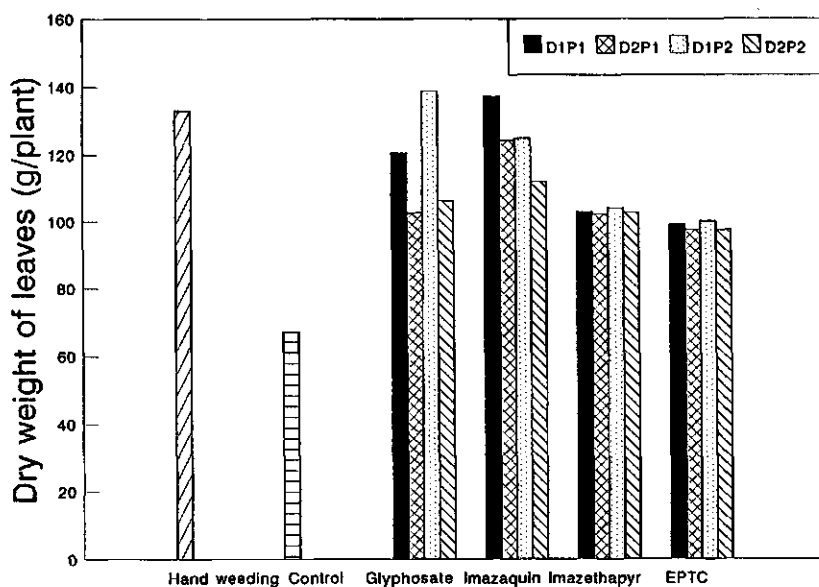
Glyphosate:D1 0.5 and D2 1.0 kg a.i./ha;Imazaquin:D1 0.01 and D2 0.02 kg a.i./ha  
 Imazethapyr:D1 0.015 and D2 0.03 kg a.i./ha;EPTC:D1 0.5 and D2 1.0 kg a.i./ha  
 D1:Lower dose; D2:Higher dose;P1:30 and P2:60 days after transplanting; LSD (0.05): 8.1

Fig. 5. Effect of herbicides on the plant height of tobacco at harvest.



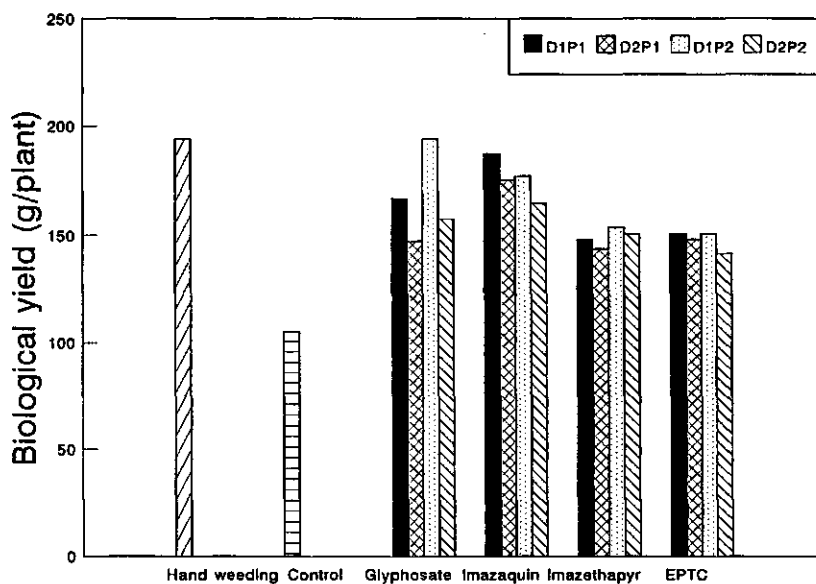
Glyphosate:D1 0.5 and D2 1.0 kg a.i./ha;Imazaquin:D1 0.01 and D2 0.02 kg a.i./ha  
 Imazethapyr:D1 0.015 and D2 0.03 kg a.i./ha;EPTC:D1 0.5 and D2 1.0 kg a.i./ha  
 D1:Lower dose; D2:Higher dose;P1:30 and P2:60 days after transplanting; LSD (0.05): 8.4

Fig. 6. Effect of herbicides on the dry weight of leaves of infested tobacco plants at harvest.



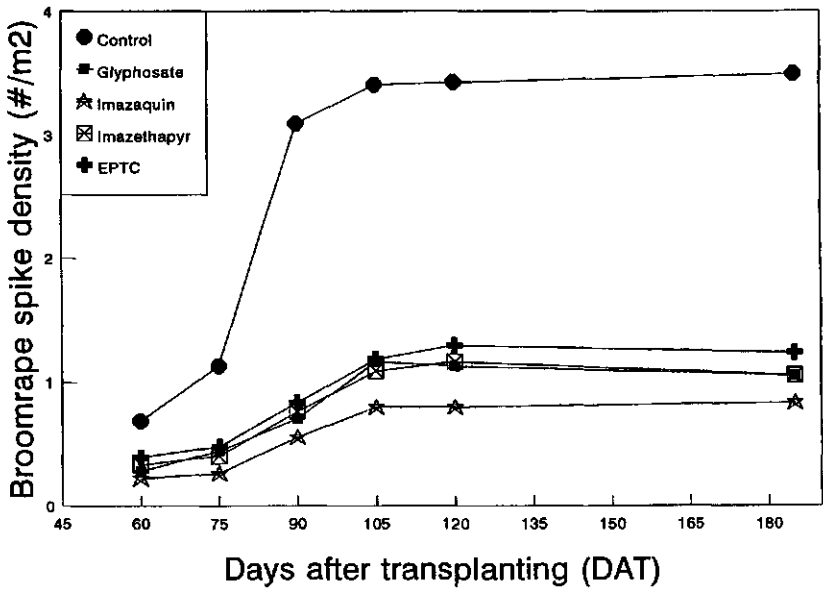
Glyphosate:D1 0.5 and D2 1.0 kg a.i./ha;Imazaquin:D1 0.01 and D2 0.02 kg a.i./ha  
 Imazethapyr:D1 0.015 and D2 0.03 kg a.i./ha;EPTC:D1 0.5 and D2 1.0 kg a.i./ha  
 D1:Lower dose; D2:Higher dose;P1:30 and P2:60 days after transplanting; LSD (0.05): 6.6

Fig. 7. Effect of herbicides on the dry weight of leaves of non-infested tobacco plants at harvest.



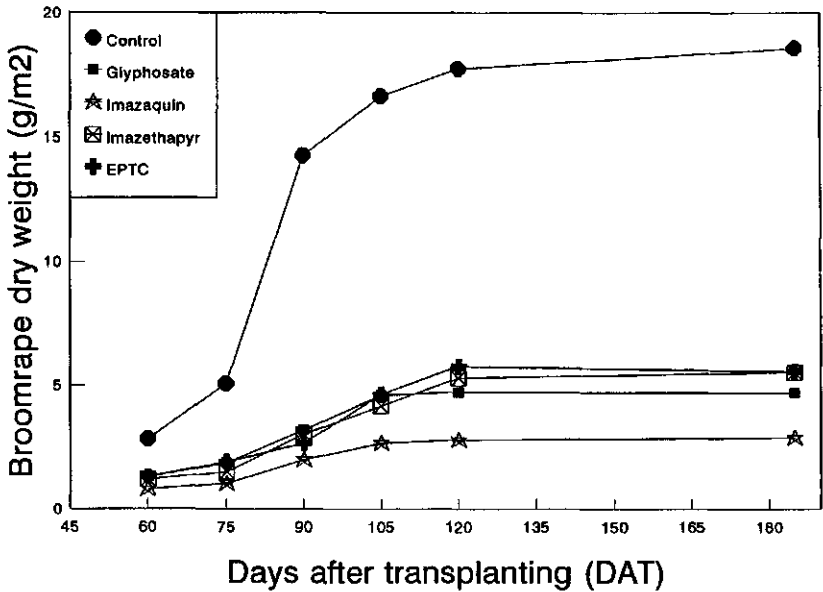
Glyphosate:D1 0.5 and D2 1.0 kg a.i./ha;Imazaquin:D1 0.01 and D2 0.02 kg a.i./ha  
 Imazethapyr:D1 0.015 and D2 0.03 kg a.i./ha;EPTC:D1 0.5 and D2 1.0 kg a.i./ha  
 D1:Lower dose; D2:Higher dose;P1:30 and P2:60 days after transplanting; LSD (0.05): 10.2

Fig. 8. Effect of herbicides on the biological yield of tobacco at harvest.



Glyphosate @ 1.0 kg a.i./ha at 30 DAT; Imazaquin @ 0.02 kg a.i./ha at 30 DAT  
 Imazethapyr @ 0.030 kg a.i./ha at 30 DAT; EPTC @ 1.0 kg a.i./ha at 30 DAT; Harvest: 185 DAT  
 LSD (0.05): 60 DAT: 0.15; 75 DAT: 0.16; 90 DAT: 0.34; 105 DAT: 0.39; 120 DAT: 0.54; Harvest: 0.58

Fig. 9. Effect of herbicides on the broomrape spike density in tobacco crop over

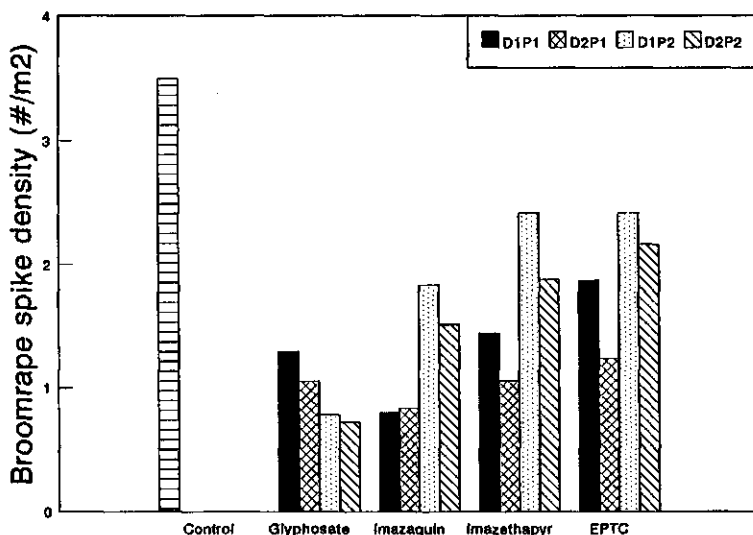


Glyphosate @ 1.0 kg a.i./ha at 30 DAT; Imazaquin @ 0.02 kg a.i./ha at 30 DAT  
 Imazethapyr @ 0.030 kg a.i./ha at 30 DAT; EPTC @ 1.0 kg a.i./ha at 30 DAT; Harvest: 185 DAT  
 LSD (0.05): 60 DAT: 0.78; 75 DAT: 0.86; 90 DAT: 1.68; 105 DAT: 2.15; 120 DAT: 2.17; Harvest: 2.69

Fig. 10. Effect of herbicides on the broomrape dry weight in tobacco crop over time.

The effect of herbicides on the broomrape population in tobacco crop at harvest is illustrated in Figure 11. The broomrape spike density was significantly reduced at higher concentrations of herbicide treatments at 30 or 60 DAT compared to the concentrations at their lower levels. Glyphosate at 30 or 60 DAT and imazaquin, imazethapyr and EPTC at 30 DAT at their lower or higher concentrations reduced broomrape spikes greatly, while EPTC and imazethapyr at 60 DAT at their lower concentration did not reduce broomrape spikes satisfactorily. In general, all herbicides at their higher concentrations reduced broomrapes significantly more than at their lower concentrations. Highest numbers of broomrape were counted in the untreated tobacco. However, all herbicides effectively reduced the broomrape spike density at their lower concentrations. The trends were similar for the dry weight of broomrapes at harvest (Fig. 12).

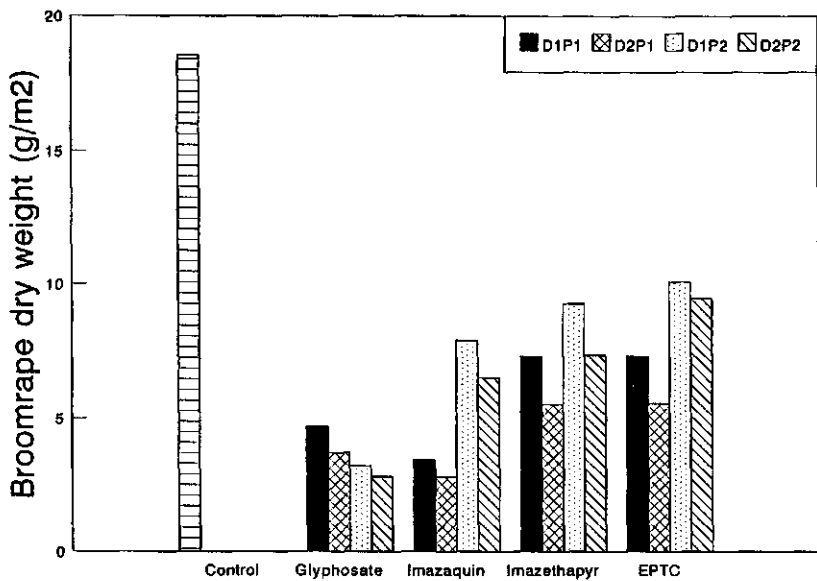
The effect of herbicides on weed (broomrape) index values in the tobacco crop at harvest is depicted in Figure 13. Extremely low weed index values were found for glyphosate at 0.50 kg a.i./ha applied at 60 days after transplanting and imazaquin at 0.01 kg a.i./ha applied at 30 days after transplanting meaning that economical yields of tobacco were on par with the hand weeding treatment. 17 - 37% reduction in the economical yield of tobacco was observed in all herbicides at their higher concentrations at 30 or 60 days after transplanting tobacco. The highest weed index value was calculated for the control treatment indicating 58% loss of economical yield of tobacco due to the infestation of broomrapes alone.



Glyphosate: D1 0.5 and D2 1.0 kg a.i./ha; Imazaquin: D1 0.01 and D2 0.02 kg a.i./ha  
 Imazethapyr: D1 0.015 and D2 0.03 kg a.i./ha; EPTC: D1 0.5 and D2 1.0 kg a.i./ha; Harvest: 185 DAT  
 D1: Lower dose; D2: Higher dose; P1: 30 and P2: 60 days after transplanting; LSD (0.05): 0.58

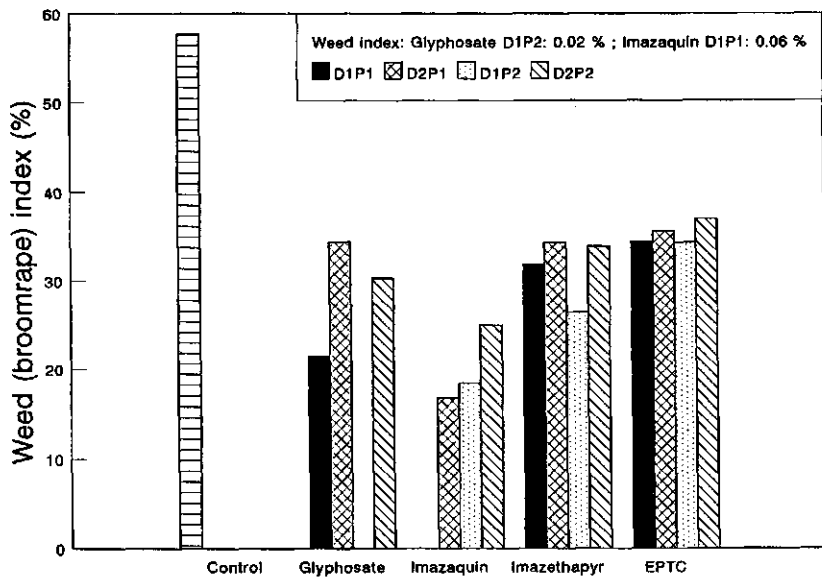
Fig. 11. Effect of herbicides on the broomrape spike density in tobacco crop at harvest.





Glyphosate:D1 0.5 and D2 1.0 kg a.i./ha;Imazaquin:D1 0.01 and D2 0.02 kg a.i./ha  
 Imazethapyr:D1 0.015 and D2 0.03 kg a.i./ha;EPTC:D1 0.5 and D2 1.0 kg a.i./ha; Harvest: 185 DAT  
 D1:Lower dose; D2:Higher dose;P1:30 and P2:60 days after transplanting; LSD (0.05): 2.69

Fig. 12. Effect of herbicides on the broomrape dry weight in tobacco crop at harvest.



Glyphosate:D1 0.5 and D2 1.0 kg a.i./ha;Imazaquin:D1 0.01 and D2 0.02 kg a.i./ha  
 Imazethapyr:D1 0.015 and D2 0.03 kg a.i./ha;EPTC:D1 0.5 and D2 1.0 kg a.i./ha  
 D1:Lower dose; D2:Higher dose;P1:30 and P2:60 days after transplanting

Fig. 13. Effect of herbicides on weed (broomrape) index in tobacco at harvest.

## 5.4 Discussion

Herbicides may be used to control and thereby prevent reproduction of parasitic weeds (Foy et al., 1989; Sauerborn et al., 1989; Garcia-Torres et al., 1994). Their use is limited, their major constraints being availability, cost, and the equipment and technology required for their use, particularly in the Third World countries (Eplee and Norris, 1995).

After a first screening of 15 chemicals we excluded the majority from further testing, leaving four for further study, viz. glyphosate, imazaquin, imazethapyr and EPTC. All of these were found to strongly reduce broomrape numbers and dry weight, and resulted in increased tobacco yields compared to the non-treated control.

Glyphosate, a systemic herbicide can be effectively used in controlling broomrape either before or after emergence of the shoots. When sprayed on the host plant it is translocated to the parasite, which is killed. It was reported to be effective in faba bean, controlling *O. crenata* (e.g. Halila, 1988; Saber et al., 1994), and *O. aegyptiaca* (Sauerborn et al., 1989) as well as in sunflower (Castejon-Muñoz et al., 1990). The concentrations used by these authors ranged from less than 0.050 to 0.090 kg/ha. Its phytotoxicity to crops is a limiting factor. Some hosts viz. broad bean, cabbage, carrot and vetch can tolerate glyphosate, but tomato, eggplant and pea are extremely sensitive (Jacobsohn and Levy, 1986). Lolas (1994) noticed that glyphosate and sulfosate each at 0.2 + 0.3 kg a.i./ha and imazaquin at 0.01 + 0.10 kg a.i./ha at 40 and 60 days after transplanting gave excellent control of *Orobancha ramosa* with no adverse effect on tobacco growth and yield; however, 9% formulation of glyphosate damaged the crop. In the present study, we found the best results when glyphosate was applied at 60 DAT at a rate of 0.50 kg a.i./ha. There was some scorching of leaves at the growing tip of tobacco, but plants recovered later. These data point to a sufficient degree of tolerance of tobacco to glyphosate to allow its use for effective broomrape control.

Effectivity of imazaquin and imazethapyr in broomrape control were tested e.g. by Saber et al. (1994). They found that two times 0.04 kg/ha of imazaquin or imazethapyr effectively controlled *O. crenata* in faba bean fields. Previous results were summarized by Parker and Riches (1993). Our data indicated that even lower concentrations of imazaquin of 0.01 kg/ha applied at 30 DAT were sufficient for broomrape control in tobacco, whereas imazethapyr was less effective. No reports were found in the literature on the use of EPTC in broomrape control. Our data indicate also that this herbicide is less effective than the other three; it does not seem to deserve further work.

Recent work by Garcia-Torres and Lopez-Granados (1991) and Garcia-Torres et al. (1994) using imidazolinones and sulphonylure as applied at pre-emergence

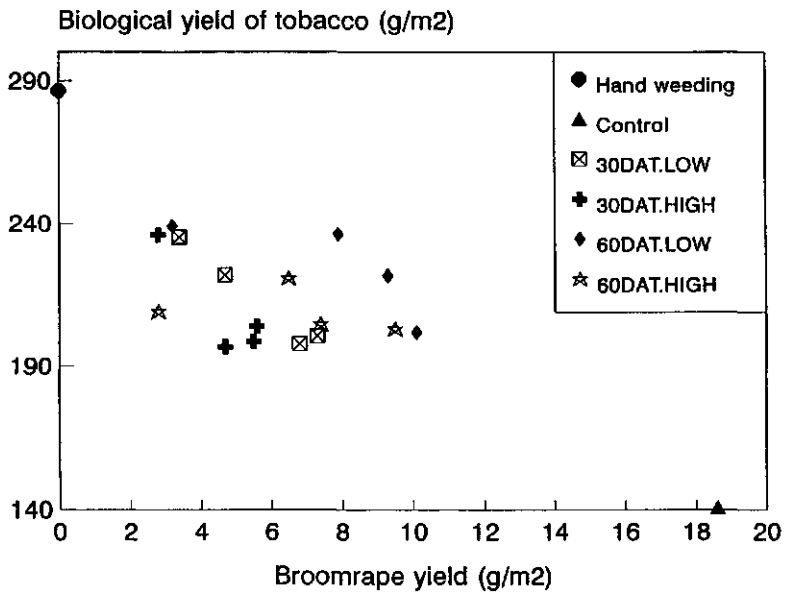


Fig. 14. Relation between broomrape yield and biological yield of tobacco.

showed promising results in controlling broomrapes in faba bean and sunflower. Their efficacy in tobacco needs further testing.

In many of the above studies concentration and timing of herbicide application appeared to be crucial. Concentration does not only concern susceptibility of the weed itself, but also sensitivity of the crop. This makes that several herbicides, systemic herbicides in particular, which can be very effective in general weed control, cannot be used to control broomrapes. The data indicate that tobacco is relatively resistant in this respect.

Although the relation between broomrape yield and tobacco yield at final harvest was highly significant for the entire set of data ( $r^2 = 0.63$ ;  $n = 18$ ), this was mainly due to the extreme values for the untreated control and the hand weeding (Fig. 14). When the latter two points were deleted the relation between broomrape was not significant. This means that concentration did not consistently affect the relation between these two parameters. Timing, however, seems to be an important factor, except for glyphosate (Fig. 14).

Castejon-Muñoz et al. (1990) found that precisely timed and repeated application of very low doses of glyphosate resulted in high levels of control.

Apparently this method is better in reaching the successive broomrapes, attaching to the host. Although their development may continue as long as living roots are available, the effect of the late-established broomrapes seems to be limited, so that spraying can be limited to two or three times, early in the host life cycle. Since very low doses appeared to be sufficient, this procedure deserves further testing in tobacco. Since the effectivity of these control methods may depend on environmental conditions, the tests should be done in the local situation.

### Acknowledgements

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## Chapter 6

## Chapter 6

**REDUCTION OF INFESTATION OF BROOMRAPE ON TOBACCO BY METABOLIC INHIBITION USING MALEIC HYDRAZIDE**G.N. DHANAPAL<sup>1</sup> and P.C. STRUIK<sup>2</sup>

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**Summary**

The effectiveness of maleic hydrazide (1, 2 - dihydropyridazine - 3, 6 - dione) in controlling *Orobanche cernua* and its effect on growth and yield of bidi tobacco were evaluated at the Agricultural Research Station, Nipani, India during 1994-95. Effective control of *Orobanche cernua* without adverse effects on tobacco growth and yield proved difficult to achieve with a single foliar application of maleic hydrazide. Application of MH at 0.25 - 0.75 kg a.i./ha applied at 30 or 40 days after transplanting bidi tobacco resulted in about 75% fewer *Orobanche cernua* spikes. At 0.50 and 0.75 kg a.i./ha MH inhibited the parasite satisfactorily but reduced the yields of bidi tobacco due to its phytotoxicity. Best results were obtained with 0.25 kg a.i./ha MH, but even this treatment only gave tobacco yields similar to the ones obtained with hand weeding.

**6.1 Introduction**

Broomrape (*Orobanche cernua* Loefl.) is a noxious parasitic weed on tobacco in India. Broomrape disrupts physiological and metabolic processes in host plants (Lolas, 1986), especially increases respiration (Singh and Singh, 1977; De la Harpe et al., 1981) thus reducing economical yield of tobacco by 40 - 45% (Dhanapal and Struik, 1996). Various control methods such as crop rotation, deep ploughing, use of fertilizers, growing of trap crops, solarization, use of resistant varieties and chemical control have been evaluated (Pieterse, 1979; Foy et al., 1989), but none of the methods so far appeared to be satisfactory. Large numbers of chemicals



have been tested against broomrape but only a few have been reported to be effective or partially effective under field conditions (Lolas, 1986; Foy et al., 1989; Nemli et al., 1989; Kotoula-Syka and Elephtherohorinos, 1991; Lolas, 1994).

MH sprayed on tobacco leaves effectively inhibited the development of *Orobanchae aegyptiaca* and *Orobanchae ramosa* and imparted immunity to the host plants (Evtushenko et al., 1973). Lolas (1986) reported satisfactory control of *Orobanchae ramosa* by the application of MH at 0.7 kg/ha applied at 40 and 60 days after transplanting tobacco with some crop injury. Mazahari et al., 1991 suggested two applications of MH and glyphosate at 40 and 60 days after transplanting at 0.7 + 0.7 kg a.i./ha and 0.05 + 0.10 kg a.i./ha resulted in 87 - 89% and 52 - 64% control of *Orobanchae aegyptiaca* respectively. Lolas (1994) observed good control of *Orobanchae ramosa* by maleic hydrazide at 0.45 + 0.41 kg a.i./ha.

A field study was conducted with maleic hydrazide, a systemic herbicide, applied as a metabolic inhibitor at different levels and at different dates to optimise the application that would allow control of the parasite with minimum growth inhibition of the tobacco crop.

## 6.2 Materials and Methods

A field experiment was conducted at the Agricultural Research Station, Nipani, India during the late kharif (spring; rainy period) and rabi (winter) seasons in 1994-95. The research station is located at 16° 2' North latitude and 74° 2' East longitude, at an altitude of 610 metres above sea level, on a black clay loam soil. The pH of the soil is 8.3. The average rainfall is 720 mm. Relative humidity fluctuates between 38% and 85%; the mean monthly maximum temperature ranges from 29.8 °C (December) to 37.5 °C (April) and the mean monthly minimum temperature ranges from 13.6 °C (December) to 21.9 °C (May).

The effects of maleic hydrazide (1, 2 - dihydropyridazine - 3, 6 - dione) at different concentrations and times of application on the parasite and on the growth and yield parameters of tobacco were investigated. For this purpose, a field naturally infested with *Orobanchae cernua* was selected. A bulk crop bidi tobacco was grown on the experimental plot during 1993-94 as a preceding crop and later the plot was kept fallow for a period of six months. The experiment consisted of eight treatment combinations: maleic hydrazide at the rate of 0.25 kg a.i./ha (416 ppm), 0.50 kg a.i./ha (833 ppm), and 0.75 kg a.i./ha (1249 ppm) each at 30 and 40 days after transplanting tobacco (i.e. when *Orobanchae* usually starts to manifest itself), a hand weeding treatment and the control. The experiment was laid out in a randomised block design with four replications. The plot size was 8 × 4.5 m<sup>2</sup>, but all the biometric observations were recorded on 6 × 3 m<sup>2</sup>.

Seedlings of cv. Anand-119 were raised under optimal conditions until they

were 40 days old (plant height approx. 10 - 12 cm). After a thorough preparation of the land and some showers, the bidi tobacco was transplanted on September 5, 1994 at a row to row distance of 1.0 m and a plant to plant distance within rows of 0.75 m in 36 m<sup>2</sup> plots. Thus, the plant density was 1.33/m<sup>2</sup>. Cultural practices and crop protection measures other than *Orobanche* control were as appropriate for the location. Maleic hydrazide at different concentrations was sprayed on October 5, 1994 (30 days after transplanting) and on October 15, 1994 (40 days after transplanting) by means of a backpack sprayer, using 0.06 litres of water per m<sup>2</sup>. In general the *Orobanche cernua* emergence above-ground takes place after 35 - 40 days after transplanting tobacco under Indian soil conditions; hence spraying of MH at 30 and 40 after transplanting were selected. In the hand weeding treatment, all the broomrapes were hand pulled as and when they emerged above ground. The crop was harvested at 185 days after transplanting.

Non-destructive biometric observations included plant height and number of leaves per plant from five randomly selected plants at 45, 60, 90, 120 days after transplanting and at harvest. Fresh weight of leaves (g/plant), dry weight of leaves and dry weight of stem (g/plant), and total dry matter (g/plant) were assessed from two randomly selected "infested" and "non-infested" plants per plot at 60, 90, 120 and 185 days after transplanting. The term "infested" refers to tobacco plants on which broomrape spikes were seen above ground, whereas, "non-infested" refers to tobacco plants on which the broomrape spikes were not seen above ground, although they might be infected below ground.

All the *Orobanche cernua* spikes present in the net plot area (18 m<sup>2</sup>) were counted on 45, 60, 75, 90, 105, 120 and 185 days after transplanting. The parasitic spikes were collected from an area of 1.0 m<sup>2</sup> from each net plot area at 60, 75, 90, 105, 120 and 185 days after transplanting. The plant samples were oven dried at 70 °C and the dry weights were recorded. The removal of spikes was common in all the treatments at different stages, therefore the comparison is valid.

In all figures, the control treatments are considered as treatments with a MH concentration of 0 kg a.i./ha.

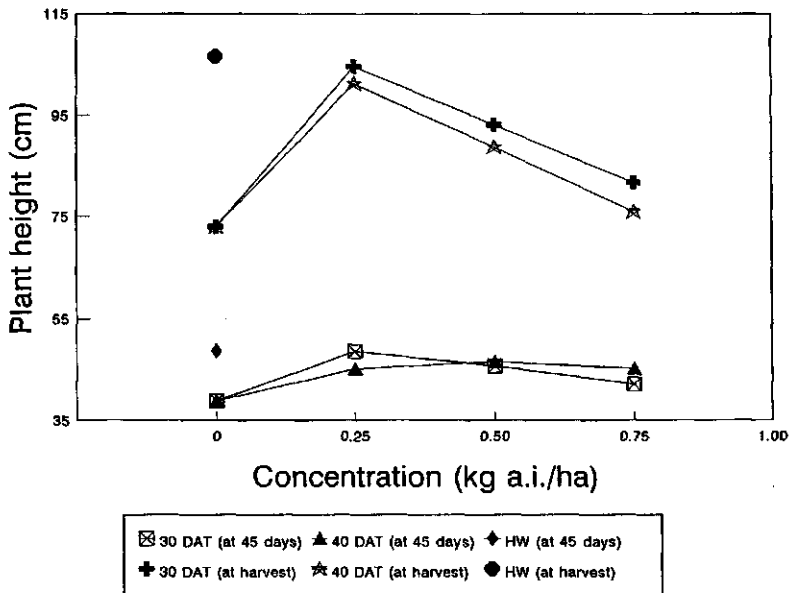
#### Statistical procedures

The data on number and dry weight of broomrape spikes at different dates were square root transformed, to obtain homogeneity of variance. Fischer's method of 'Analysis of Variance' was applied for the interpretation of the data. The level of significance used in 'F' and 't' tests was P=0.05. The values of 'F' and 't' and least significant differences (LSD) were calculated following the method outlined for a Randomised Block Design by Panse and Sukhatme (1967).

### 6.3 Results

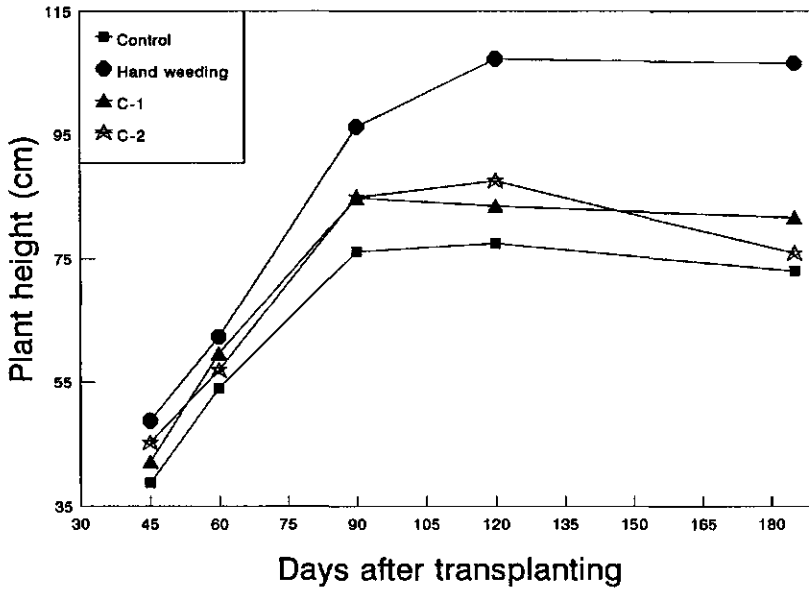
The plant height of tobacco at 45 days after transplanting was already significantly reduced by maleic hydrazide at 0.50 kg a.i./ha and 0.75 kg a.i./ha applied at 30 or 40 days after transplanting tobacco as compared to the hand weeding treatment (Fig. 1). The plant height was reduced even more in the control treatment by the broomrape infection. At harvest the effect of maleic hydrazide on the tobacco plant height was even more obvious (Fig. 1). The plant height of tobacco in the MH treatment with 0.25 kg a.i./ha applied at 30 or 40 days after transplanting was similar to the plant height in the hand weeding treatment. At 0.50 and 0.75 kg a.i./ha applied at 30 or 40 days after transplanting tobacco, the plant height was significantly reduced indicating the toxic effect of maleic hydrazide at higher concentrations. However, the growth inhibiting effect of the broomrape in the control was also very large.

It was also noticed that higher concentrations of maleic hydrazide caused apical leaves to be abnormally narrow and elongated, serrated and wavered at the leaf margins, showing necrosis and interveinal bleaching; tobacco plant growth was stunted. The effect of maleic hydrazide at 0.75 kg a.i./ha applied at 30 or 40 days after transplanting on the plant height of tobacco over time in comparison with the untreated and hand weeding controls is illustrated in Figure 2 mainly to indicate the



DAT: Days after transplanting; 0: Control; HW: Hand weeding  
 LSD (0.05): at 45 DAT: 3.0; at harvest: 9.7

Fig. 1. Effect of maleic hydrazide on tobacco plant height at 45 days after planting and at harvest.

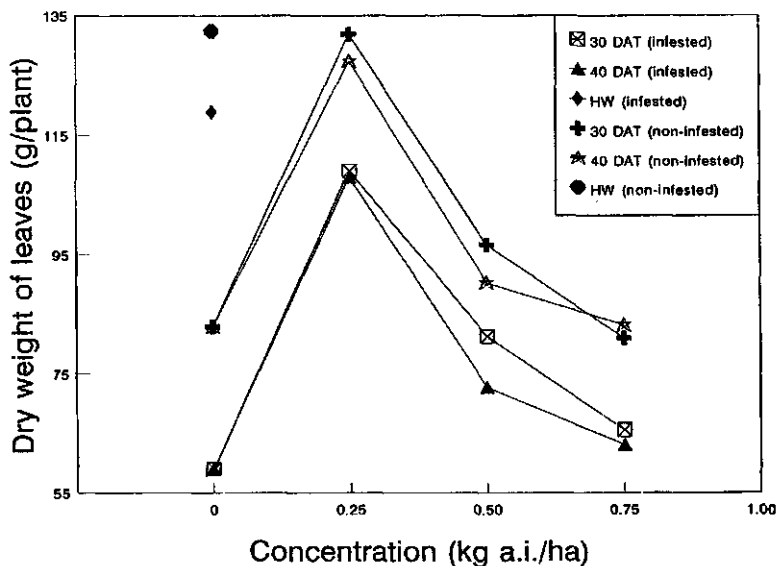


C-1: Maleic hydrazide @ 0.75 kg a.i./ha at 30 DAT; Harvest: 185 DAT  
 C-2: Maleic hydrazide @ 0.75 kg a.i./ha at 40 DAT; DAT: Days after transplanting  
 LSD (0.05): at 45 DAT: 3.0; 60 DAT: 3.3; 90 DAT: 4.8; 120 DAT: 5.0; Harvest: 9.7

Fig. 2. Effect of maleic hydrazide on the plant height of tobacco over time.

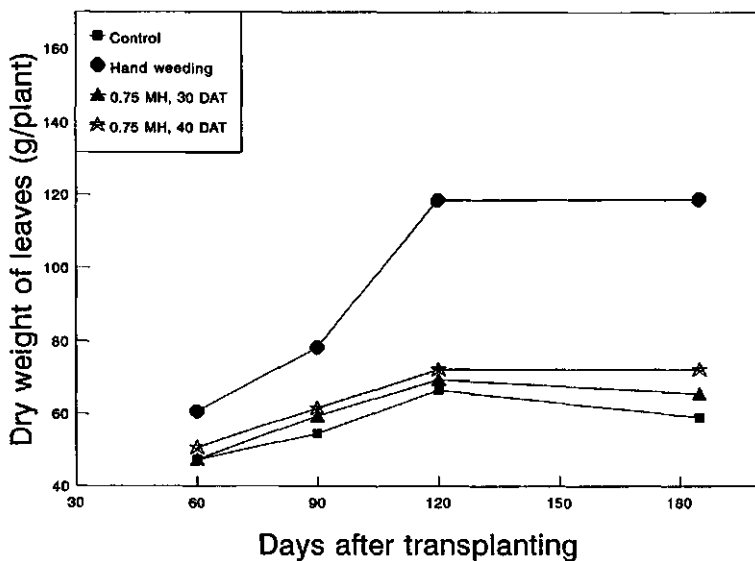
its toxic effects. The untreated tobacco remained small, whereas the hand weeded crop grew much more vigorously. The high concentrations of maleic hydrazide showed intermediate growth; there was no effect of date of treatment; at harvest, plant height was similar to the control treatment.

Dry weight of leaves (the harvestable product) of "infested" tobacco plants was highest in the hand weeding treatment followed by maleic hydrazide at 0.25 kg a.i./ha applied at 30 or 40 days after transplanting (Fig. 3). The dry weight of leaves was lower in the treatments with 0.50 and 0.75 kg a.i./ha maleic hydrazide applied at 30 or 40 days after transplanting than in the hand weeding treatment. However, lowest dry weight of tobacco leaves was obtained in the control treatment. The trends in dry weight of leaves of "non-infested" tobacco plants were similar (Fig. 3). Higher dry weights of tobacco leaves in "non-infested" tobacco plants may be attributed to the controlling of broomrape structures below-ground. Again, the best maleic hydrazide treatment was almost as good as the hand weeding treatment. Higher concentrations of maleic hydrazide reduced yields compared to the hand weeding treatment both in "infested" and "non-infested" tobacco plants (Figs. 4a and 4b).



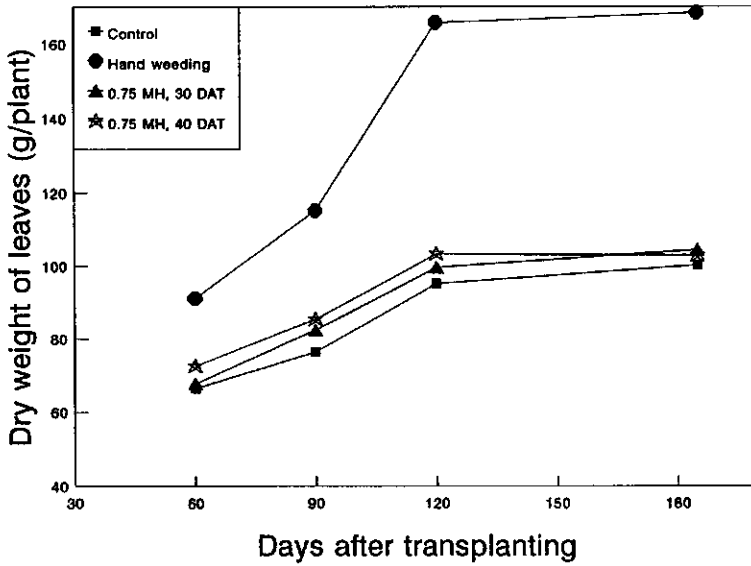
DAT: Days after transplanting ; 0: Control ; HW: Hand weeding  
 LSD (0.05): Infested: 6.7; Non-infested: 8.3

Fig. 3. Effect of MH on the dry weight of tobacco leaves (infested & non-infested plants) at harvest.



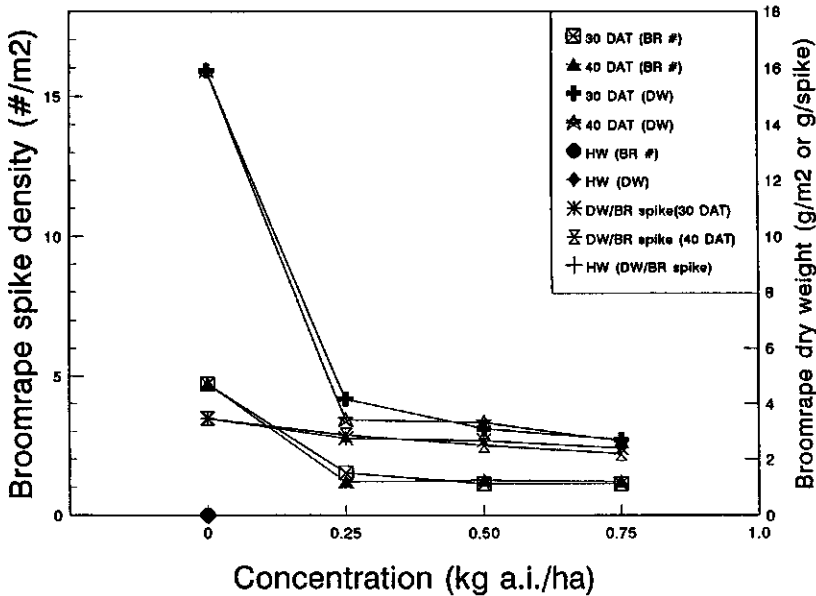
DAT: Days after transplanting; Harvest: 185 DAT  
 LSD (0.05): 60 DAT: 6.2; 90 DAT: 8.3; 120 DAT: 6.1; Harvest: 6.7

Fig. 4a. Effect of maleic hydrazide on the tobacco leaf dry weight (infested plants) over time.



DAT: Days after transplanting; Harvest: 185 DAT  
 LSD (0.05): 60 DAT: 5.1; 90 DAT: 8.9; 120 DAT: 7.0; Harvest: 8.3

Fig. 4b. Effect of maleic hydrazide on the tobacco leaf dry weight (non-infested plants) over time.



DAT: Days after transplanting; BR #: Broomrape number; DW: Dry weight; HW: Hand weeding  
 0: Control; LSD (0.05): Broomrape number: 0.016; Broomrape dry weight: 0.37

Fig. 5. Effect of maleic hydrazide on the broomrape number and dry weight at harvest.

High numbers of broomrapes were found in the control treatment (Fig. 5) at harvest. The significant inhibition of *Orobancha cernua* was more at 0.50 and 0.75 kg a.i./ha maleic hydrazide applied at 30 or 40 days after transplanting tobacco than in the lowest dose. In the hand weeding control, virtually no broomrape was seen above ground, because they were weeded out as and when they emerged out of the soil. Highest dry weight of broomrapes were also obtained in the control treatment at final harvest (Fig. 5). Significantly lower dry weight of broomrape was recorded in the maleic hydrazide treatment at 0.25 kg a.i./ha applied at 30 or 40 days after transplanting as compared to the control. Effects of treatments on broomrape yields were mainly caused by their effects on spike density and not on individual spike weight (Fig. 5). Maximum inhibition of *Orobancha cernua* was noticed in the treatments with maleic hydrazide at 0.75 and 0.50 kg a.i./ha applied at 30 or 40 days after transplanting tobacco.

#### 6.4 Discussion

Maleic hydrazide, a systemic herbicide, can be effectively used to control broomrapes selectively in some crops (Foy et al., 1989). As a post-emergence foliar herbicide, maleic hydrazide can be sprayed on crop plants so that it is translocated through the host plant and into the parasite, thereby killing the parasite. The presence of a parasite can alter the rate of important host metabolic functions, such as photosynthesis, respiration and the uptake of water and solutes (Graves, 1995).

The results indicate that the concentration applied is crucial; the tobacco plant height was greatly reduced at higher concentrations of maleic hydrazide, due to its toxic effect. This toxic effect was almost as detrimental to the tobacco plant as the infection by *Orobancha*. The plant height of tobacco at 0.25 kg a.i./ha maleic hydrazide was similar to the height of the hand weeding treatment indicating that the best positive effect of maleic hydrazide was still well below the potential. The time of application, however, was not critical in the range investigated.

Highest dry weight of leaves both in "infested" and "non-infested" tobacco plants was obtained in the hand weeding treatment followed by maleic hydrazide at 0.25 kg a.i./ha applied at 30 or 40 days after transplanting, since it reduced the density of *Orobancha cernua* spikes throughout the growing season of bidi tobacco with only limited crop injury. These results confirm the findings of Lolas (1986, 1994). Before emergence, however, *Orobancha* has already affected tobacco growth. The fact that the best maleic hydrazide treatment could not increase tobacco yields compared to the hand weeding control suggests that even at that low concentration some crop injury occurred.

The inhibition of *Orobancha cernua* was maximum in maleic hydrazide at 0.75

and 0.50 kg a.i./ha applied at 30 or 40 days after transplanting tobacco followed by maleic hydrazide at 0.25 kg a.i./ha.

Our results indicate that effective control of *Orobanche cernua* in bidi tobacco without adverse effects on crop growth and yield is very difficult to achieve with a single foliar application of maleic hydrazide. Maleic hydrazide at 0.50 and 0.75 kg a.i./ha applied at 30 or 40 days after transplanting inhibited *Orobanche cernua* satisfactorily, but reduce the yields of bidi tobacco due to its phytotoxicity.

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## Chapter 7

## Chapter 7

### STUDIES ON THE POST-EMERGENCE CONTROL OF BROOMRAPE WITH NATURAL PLANT OILS

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#### Summary

The effect of natural plant oils on *Orobancha cernua* Loeﬂ. was assessed in a naturally infested tobacco field at the Agricultural Research Station, Nipani, India during 1994-95. Natural plant oils differed in their ability to kill the young broomrape spikes. Neem oil, coconut oil and sunflower oil showed quick knock-down effects on the bud part of the parasite compared to castor and niger oils. Mustard oil took more days to kill the bud.

Coconut and sunflower oils also killed the broomrape stem more quickly than niger and castor oils. Neem oil and mustard oil did not kill the stem part of the parasite. None of the oils was phytotoxic to tobacco.

#### 7.1 Introduction

*Orobancha cernua* Loeﬂ. is a serious root parasite and a major constraint to tobacco production in India. It debilitates the tobacco growth by disrupting the physiological and metabolic processes in the host plant thereby causing wilting and a ribbed appearance of leaves (Krishnamurthy, 1991). Tobacco cannot be grown economically in fields infested with broomrapes.

Swabbing natural plant oils on young broomrape spikes will kill the parasite without affecting the host plant (Krishnamurthy and Chari, 1991). This is an effective method only when it is carried out before flowering of the parasite. The method is labour-intensive, because all spikes must be treated individually and the treatment has to be repeated when new spikes emerge. However, the method requires less time than mechanically removing the spikes.

Natural plant oils as bioherbicides may play an important role in preventing the parasitic weed seed formation thereby reducing the seed bank of the parasite. Unlike chemical herbicides, these natural plant oils do not cause environmental problems; they are easily and rapidly degraded in the soil. Krishnamurthy and Chari (1991) suggested that research on the mode of action of plant oils, efficacy of plant oils and nature of death of broomrape due to application of plant oils is required.

With this background a field study was conducted to compare the effects of several natural plant oils to control *Orobanche cernua*. The main objectives of this study were to assess the extent of control of the parasite before flowering and the phytotoxic effect on tobacco leaves.

## 7.2 Materials and Methods

A field experiment was conducted at the Agricultural Research Station, Nipani, India during 1994-95 "kharif" (spring; rainy period) and "rabi" (winter) seasons. The research station is located at 16° 2' North latitude and 74° 2' East longitude, at an altitude of 610 metres above sea level, on a black clay loam soil. The pH of the soil ranges from 8.2 to 8.4. The average rainfall is 720 mm. The relative humidity fluctuates between 38% and 85%; the mean monthly maximum temperature ranges from 29.8 °C (December) to 37.5 °C (April) and the mean monthly minimum temperature ranges from 13.6 °C (December) to 21.9 °C (May). Bidi tobacco has been grown at the research station for 30 years (continuous cropping).

An experimental area naturally infested with *Orobanche cernua* in a bidi tobacco field was selected for the study. Young broomrape shoots of the following stages were identified for the study:

Stage I: broomrape spikes after 5 days of visual emergence above-ground containing only the bud part.

Stage II: broomrape spikes of 5 cm height having both bud and stem parts.

Natural plant oils viz. castor oil (*Ricinus communis* L.), coconut oil (*Cocos nucifera* L.), mustard oil (*Brassica juncea* (L.) Czernjaew), neem oil (*Azadirachta indica* Juss.), sunflower oil (*Helianthus annuus* L.) and niger oil (*Guizotia abyssinica* (L. fil.) Cass.) were used for the study. The composition of these plant oils is presented in Table 1.

The following procedure was followed:

- a) 100 broomrape spikes for each stage were selected randomly in a tobacco field naturally infested with *Orobanche cernua* in an area of 50 m<sup>2</sup> and such plants were labelled.
- b) 3 drops of a plant oil were applied on the tip of the individual spike using a

Table 1. Composition of natural plant oils (adapted from Jamieson, 1943; Godin and Spensley, 1971; Rao and Rao, 1981; Wiess, 1983). Bold figures indicate the dominant fatty acid(s) for each species.

Contents (%)	Formula	Natural plant oils					
		Neem <sup>a</sup>	Coconut <sup>b</sup>	Sunflower <sup>c</sup>	Niger <sup>d</sup>	Castor <sup>e</sup>	Mustard <sup>f</sup>
Caproic acid	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	-	0.8	-	-	-	-
Caprylic acid	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	-	9	-	-	-	-
Lauric acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	-	50	-	-	-	-
Myristic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	-	18	-	0.4	-	-
Palmitoleic acid	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	-	-	-	-	-	0.5
Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	15	10	7.2	6	1	3
Linolenic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	-	-	-	1.2	0.3	14.5
Linoleic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	12	2	<b>72.5</b>	<b>52</b>	4.5	17.5
Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	<b>56</b>	7	16.2	<b>36</b>	trace	9
Ricinoleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>	-	-	-	-	<b>92</b>	-
Stearic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	17	3	4.1	4	1	1.5
Dihydroxystearic acid	C <sub>18</sub> H <sub>36</sub> O <sub>4</sub>	-	-	-	-	0.7	-
Arachidic acid <sup>g</sup>	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	little	0.2	-	0.4	0.3	8
Erucic acid	C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>	-	-	-	-	-	<b>46</b>

<sup>g</sup> Arachidic acid = Eicosenoic acid

<sup>a</sup> Neem (*Azadirachta indica* Juss.)

<sup>b</sup> Coconut (*Cocos nucifera* L.)

<sup>c</sup> Sunflower (*Helianthus annuus* L.)

<sup>d</sup> Niger (*Guizotia abyssinica* (L. fil.) Cass.)

<sup>e</sup> Castor (*Ricinus communis* L.)

<sup>f</sup> Mustard (*Brassica juncea* (L.) Czernjaew)

dropper which discharges 0.03 ml per drop (i.e. 0.09 ml per broomrape spike). 0.09 ml of oil applied on the tip of the broomrape spike was sufficient enough to percolate down to the bottom of the spike thereby killing the bud or stem or both due to its toxic effect on the parasite.

c) simultaneously 3 drops of each plant oil was applied on tobacco leaves to test the phytotoxicity.

Observations were made daily for one week on the mortality of the bud and of stem parts of broomrape spikes at both stages and on phytotoxic effects (visible signs) on the tobacco leaves.

The study was repeated in three series; each series consisted of 1200 broomrape spikes to impose different treatments, thus totalling 3600 broomrape spikes.

#### Statistical procedures

Fischer's method of 'Analysis of Variance' was applied for the analysis and interpretation of the data. The values of 'F and 't' and the critical differences were

calculated following the method outlined for one-way ANOVA by Panse and Sukhatme (1967) and the means were compared using the Student-Newman-Keuls Method (Snedecor and Cochran, 1980).

### 7.3 Results

All natural plant oils killed the bud and stem of the parasite except neem oil and mustard oil which were ineffective in killing the stem part of the parasite (Table 2). Neem oil, coconut oil and sunflower oil killed the bud part of the parasite within 3 days after application. Niger oil and castor oil took 3 - 4 days to kill the bud, and mustard oil took 5 days. The effects of the oils were very consistent for the two stages.

The effects of different oils depended on the developmental stage of the broomrape. Coconut oil and sunflower oil took 4 - 5 days to kill the stem of the

Table 2. Effect of natural plant oils on the mortality of young *Orobancha cernua* spikes (Average of three series; each series with 100 spikes for each of the treatments).

Natural plant oils	Number of days taken to kill the bud and stem of broomrape			Phytotoxicity on tobacco leaf
	Stage I <sup>#</sup>	Stage II <sup>**</sup>		
	Bud	Bud	Stem	
Neem oil	2.2a	2.2a	NC**c	No
Coconut oil	2.2a	2.4a	4.1a	No
Sunflower oil	2.3a	2.3a	4.4a	No
Niger oil	3.3b	3.4b	6.1b	No
Castor oil	3.8c	3.9c	6.1b	No
Mustard oil	4.8d	4.8d	IC*c	No
LSD (0.05)	0.36	0.40	0.35	
CV (%)	6.4	6.9	4.1	

<sup>#</sup>Stage I: broomrape spikes after 5 days of visual emergence above-ground (only bud).

<sup>\*\*</sup>Stage II: broomrape spikes of 5 cm height (both bud and stem).

\*IC: Incomplete control; stem turned weak without scorching.

\*\*NC: No control; stem remained normal.

Different letters indicate significant differences between treatments ( $P < 0.05$ ).

parasite (Table 2). Castor and niger oils killed the stem in 6 days. Mustard oil and neem oils proved ineffective in killing the stem part of the parasite; the stem of the parasite turned weak without scorching in the mustard oil treatment and neem oil did not affect the stem part of the parasite in the second stage at all. The affected stems died soon and no re-growth was observed.

There were no visible signs of phytotoxicity on tobacco leaves with any of these natural plant oils.

#### 7.4 Discussion

Plant oils kill the parasite by suffocation when the oils are applied on the tip of the young broomrape shoots (Krishnamurthy, 1992). The natural plant oils differed in their ability to kill the young broomrape spikes. Neem oil, coconut oil and sunflower oil showed quick knock-down effects on the bud part of the parasite at stage I and stage II. Mustard oil took more days to bring about the death of the bud compared to other plant oils. The effects of castor and niger oils in killing the bud part were intermediate. These results confirm the results obtained by Krishnamurthy and Chari (1991) and Krishnamurthy and Nagarajan (1991). Also fossil oils have an effect: swabbing kerosene or diesel oil on young broomrape (*Orobancha papaveris*) shoots in an opium poppy crop resulted in a 37.5 and 61.0% mortality of the parasite, respectively but kerosene and diesel oil were phytotoxic to host plants (Ramanathan, 1985).

Coconut oil and sunflower oil killed the broomrape stems quickly, whereas niger oil and castor oil took more days to kill the stem at stage II. The stem part of the parasite turned weak without scorching (incomplete killing) in the mustard oil treatment and neem oil did not affect the broomrape stem at stage II indicating these plant oils were effective only in killing the tender parts rather than the advanced growth stages of the parasite.

It is desirable to kill the entire spike, because of its high respiration (Singh and Singh, 1971; De la Harpe et al., 1981) but killing the bud portion without killing the stem part is also an option, because it prevents flowering and seed set.

The differences in effectiveness may be attributed to the chemical composition and mode of action of the plant oils concerned (Krishnamurthy and Nagarajan, 1991). The differences in efficacy of plant oils could not readily be related to differences in chemical composition as shown in Table 1. Therefore, research has to be strengthened on efficacy of plant oils, mode of action of plant oils in the parasite, nature of death of the parasite and methods of application of plant oils by developing an appropriate tool to reduce the costs.

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## Chapter 8

## Chapter 8

### BROOMRAPE CONTROL IN A CROPPING SYSTEM CONTAINING BIDI TOBACCO

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#### Summary

*Orobanche cernua* Loeffl. is a serious problem in tobacco growing in India. Including a trap crop in the rotation may help to reduce the problem. Two field experiments at the Agricultural Research Station, Nipani were conducted during 1993-94 and 1994-95 to identify potential trap crops for utilisation in a cropping system with bidi tobacco. Trap crops reduced the population of broomrape: especially sunhemp and greengram proved to be effective. Broomrape reduced economic yield of tobacco by 42% in the fallow treatment but was much less detrimental in the treatments with a trap crop. There was a close negative correlation between above-ground crop yield and the broomrape yield; broomrapes reduced the yields of their hosts much more than they invested in their own above-ground parts. Trap crops also had a positive effect on tobacco yield by other mechanisms than above-ground broomrape control, because yield increases were also observed on apparently non-infested plants. There was no systematic difference between effects of the leguminous trap crops and the non-leguminous ones.

Sunhemp and greengram are promising trap crops in a cropping system containing bidi tobacco in the areas where tobacco is grown in a long growing season during the late kharif/rabi seasons.

#### 8.1 Introduction

*Orobanche cernua* Loeffl. is a serious root parasite on the tobacco crop in India. The severity of infestation depends on the size of the seed bank of the parasite, which in itself is the result of seed production of the parasite and the natural seed

decay in the soil. In India, the tobacco crop suffers already considerably for about six weeks before the parasite appears above ground. Control of the weed after appearance therefore does not prevent damage. Given the poor performance of (systemic) herbicides for controlling *Orobanche cernua* before emergence, it is worthwhile to use biological methods.

Crop rotation with trap or catch crops, sowing date of the crop, amount of rainfall, etc. will affect the seed bank of the parasite (Linke et al., 1991). Including trap crops or catch crops in a crop rotation helps to reduce the quantity of parasitic weed seeds in the soil by inducing suicidal germination or by destroying the parasite before flowering. Thus, it is possible to reduce the number of infested crop plants.

It is known that root exudates of host and non-host crops enhance *Orobanche* seed germination (Chabrolin, 1935; Brown, 1946; Brown et al., 1951; Abu-Shakra et al., 1970). Trap crops induce the germination of broomrape seeds but are not parasitized. The germ tubes die when they are not able to reach a host and therefore the seed bank is reduced. Catch crops are susceptible host crops, which not only induce germination but are also parasitized; however, they are destroyed before the parasite flowers. The best trap or catch crops are those that have very high root densities and produce sufficient amounts of root exudates to stimulate the parasitic weed seeds (Dhanapal et al., 1996).

Hosmani (1985) claimed that cowpea (*Vigna unguiculata*), chilli (*Capsicum annum*) and pillipesara (*Phaseolus tribolus*) are the most effective trap crops in controlling *Orobanche cernua* in India. Sorghum (*Sorghum vulgare*), moth bean (*Phaseolus aconitifolius*), cowpea (*Vigna unguiculata*) and deccan hemp (*Hibiscus sabdariffa*) as trap crops stimulated the germination of *Orobanche cernua* seeds to an extent of 50 - 70% (Krishnamurthy and Chandwani, 1975).

In the absence of a suitable method to control the parasite, growing trap crops might be a useful method to reduce the problem. Therefore, a field study was conducted to identify crops capable of trapping *Orobanche cernua*, to analyse the effect on subsequent tobacco growth and to categorise the crops showing the trap crop effect for further utilisation in a cropping system containing bidi tobacco.

## 8.2 Materials and Methods

### Site characteristics

Field experiments were conducted at the Agricultural Research Station, Nipani, Karnataka State, India during the kharif (spring; rainy period) and rabi (winter) seasons in the years 1993-94 and 1994-95.

The research station is located at 16° 2' North latitude and 74° 2' East longitude, at an altitude of 610 metres above sea level, on a black clay loam having

50 - 55% silt and 20 - 25% clay (according to the Pipette method: Piper, 1960). Field capacity of the soil is 40% and the water holding capacity is 60%. The tilth has moderate drainage and is medium deep. The pH of the soil is 8.1 (1:1 soil:water, measured after 1.0 hour stirring) and the electrical conductivity is 0.28 mmhos/cm at 25 °C (Conductivity bridge method: Jackson, 1967). The soil is medium to high in organic carbon content (Wet digestion method: Jackson, 1967), available phosphorus is low (Olson's method: Jackson, 1967) and available potash is low to medium (Flame photometer method: Jackson, 1967). The content of available manganese is above the limit of deficiency and ranges from 32 - 60 ppm. Hot water soluble boron is low to optimum (0.087 - 0.650 ppm). The calcium carbonate ranges from 5.3 to 6.0% (Puri's rapid titration method: Piper, 1950). The topography of the soil is fairly uniform at the experimental site.

The area experiences dry hot summers, humid and cool monsoons and mild winters. The average rainfall is 720 mm (average over 40 years) and 60% of the yearly precipitation is received during June to August with an average of 82 rainy days per year. Relative humidity fluctuates between 38% and 85% (average of 14 years). Mean monthly maximum temperature ranges from 29.8 °C (December) to 37.5 °C (April) and mean monthly minimum temperature ranges from 13.6 °C (December) to 21.9 °C (May) (average of 14 years).

#### Experimental set-up

Trap crops were tested for their effects on the incidence of *Orobanche cernua* in the succeeding bidi tobacco and also on the growth and yield parameters of tobacco. The treatments were carried out in a rotation of tobacco - (fallow) - trap crop - tobacco.

A bulk crop bidi tobacco was grown during 1992-93 as a preceding crop on the experimental plot and later on the land was kept fallow for a period of five months. During 1993-94 (Experiment 1), treatments consisted of five crops viz., sunhemp (*Crotalaria juncea*), greengram (*Vigna radiata*), blackgram (*Vigna mungo*), sesamum (*Sesamum indicum*), soybean (*Glycine max*), and fallow (control). The term 'fallow' means that no trap crop was grown in the treatment for 45 days; later on the land preparation and other cultural operations were carried out in the same way as for trap crop treatments before transplanting tobacco. The experiment was laid out in a randomised block design with four replications. The plot size was 8 m × 6 m, all observations were recorded in a net plot of 6 m × 4.5 m.

After a lapse of 8 months, during the 1994-95 kharif and rabi seasons, a similar experiment was carried out at the same site on a spot where no other crop had been grown in 1993-94 (Experiment 2). Treatments consisted of the trap crops sunhemp (*Crotalaria juncea*), greengram (*Vigna radiata*), redgram (*Cajanus cajan*), pea (*Pisum sativum*) and sunflower (*Helianthus annuus*), and a fallow treatment (no

crop). Plot sizes were the same as in Experiment 1. The effects of the treatments on the infection of bidi tobacco by *Orobanche cernua* were tested by growing a tobacco crop after decomposition of the trap crops in both experiments.

### Cultural practice

After the harvest of the bulk tobacco crop in February 1993, stubbles were collected and clod crushing and harrowing were done to realize a fine tilth. In Experiment 1, after some showers the different trap crops were sown on 23.06.1993 at a row distance of 30 cm. These trap crops were pulled out by hand and incorporated into the soil in the same plot on 06.08.1993, 45 days after sowing. Fertilizers at the rate of 125 kg N/ha in the form of ammonium sulphate, 27.5 kg P/ha in the form of single super phosphate and 31.1 kg K/ha in the form of sulphate of potash were applied at the time of incorporating the trap crops into the soil. Fifteen days later, after allowing the trap crops to decompose thoroughly, furrows were opened with the help of a pair of bullocks at a distance of 1.0 m apart and healthy tobacco seedlings (variety: Anand-119) of 40 days old, raised in seed beds under optimal conditions, were transplanted at a within row distance of 0.75 m on 20.08.1993. Plants that did not survive transplanting were replaced at 10 days after transplanting. Plant density was thus 1.33/m<sup>2</sup>.

In Experiment 2, the trap crops were sown on 18.06.1994, hand pulled and incorporated into the soil on 01.08.1994 (i.e. 45 days after sowing). Twenty two days later, the furrows were opened by a pair of bullocks at a distance of 1.0 m apart and healthy seedlings of 38 days old (raised in beds of 1.5 × 1.0 × 0.10 m under optimum conditions) were transplanted at a within-row distance of 0.75 m on 23.08.1994. Plants that did not survive transplanting were replaced on 01.09.1994. The final plant density was 1.33/m<sup>2</sup>. At appropriate times, ridomyl 72 w.p. (metaloxyl M-Z) at a rate of 1.0 g/litre (600 litres/ha) was used against the damping off disease, acephate at a rate of 1.0 g/litre (600 litres/ha) to control aphids, and endosulfan at a rate of 2 g/litre (600 litres/ha) against cutworms and leaf eating caterpillars. Urea spray at a rate of 2.5 g/litre (600 litres/ha) was also applied on the 20th day after sowing.

The tobacco crop was topped at 50 days after transplanting and desuckered at weekly intervals in order to encourage formation of tillers and enlargement of tobacco leaves. The tobacco crop grown during 1994-95 took 185 days to mature and was harvested on 17.01.1995. The leaves were harvested in the morning hours and kept in respective plots for air-curing for 6 - 7 days.

The cultural practices in both experiments are summarized in Table 1.

### Observations

Above-ground fresh yields of trap crops were recorded before incorporation.

Table 1. Scheme of rotation and main cultural practices.

Season	Crop	Cultural practice
<u>Experiment 1</u>		
1992-93	Bidi tobacco	Harvested in February, 1993 Soil tillage
1993-94	Trap crops	Sown on 23.06.1993 Harvested on 06.08.1993
1993-94	Bidi tobacco Water logging	Planted on 20.08.1993 Second half of October, 1993
<u>Experiment 2</u>		
1992-93	Bidi tobacco	Harvested in February, 1993
1993-94	Fallow	
1994-95	Trap crops	Sown on 18.06.1994 Harvested on 01.08.1994
1994-95	Bidi tobacco	Planted on 23.08.1994 Harvested on 17.01.1995

In Experiment 1, the broomrape population was recorded at 45 days after transplanting. Thereafter, heavy and continuous rains after 55 - 60 days after transplanting tobacco created waterlogged conditions for about 8 - 10 days. Water logging is deleterious to broomrape (Zahran, 1978; Mohamed-Ahmed and Drennan, 1994). All *Orobanche cernua* plants were snubbed and after a lapse of 85 - 90 days no single broomrape spike could be found. Also tobacco is very sensitive to water logging and the damage to the crop was almost 100%. Thus, Experiment 1 of 1993-94 was vitiated except for the early observations.

In Experiment 2, biometric observations were recorded both for the parasite and the host plant. All *Orobanche cernua* spikes present in the net plot area (27 m<sup>2</sup>) were counted on 60, 75, 90, 105 and 120 days after transplanting and at harvest (day 185 after planting). Moreover, *Orobanche cernua* spikes were collected from a random area of 1.0 m<sup>2</sup> from each plot (within the net plot area) on 60, 75, 90, 105 and 120 days after transplanting and at harvest. The spikes were air-dried and then oven-dried at 70 °C and the dry weights were recorded. At harvest, all broomrape spikes present in the net plot area were pulled out and dried in the sun for a week and their dry weight was recorded.

The growth and yield of the tobacco crop were recorded at different growth

stages. Average plant height and the number of leaves per plant were recorded from five randomly selected plants on 45, 60, 90 and 120 days after transplanting and at harvest. Fresh leaf weight (g/plant) and dry weight of leaves (g/plant), dry weight of stem (g/plant) and total dry matter (g/plant) were assessed from two randomly selected "infested" or "non-infested" plants. The term "infested" refers to tobacco plants on which broomrape (*Orobancha cernua*) spikes were seen above ground. The term 'non-infested' used here refers to tobacco plants on which the broomrape spikes were not seen above ground although they might be infected below ground. Drying of the leaves and stems was done by air-curing followed by oven drying at 60 - 65 °C at 60, 90 and 120 days after transplanting and at final harvest. Length and width of leaves from two randomly selected plants both infested and non-infested with broomrape were measured; from these data leaf area was calculated by the following formula:

$$LA = \frac{L \times W \times 0.65}{100}$$

in which:

LA = leaf area (dm<sup>2</sup>)

L = length (cm)

W = width (cm)

0.65 is an empirical shape factor based on long-term experience at the research station

The leaf area index (LAI) was calculated by dividing the leaf area per plant (in m<sup>2</sup>) by the number of plants per m<sup>2</sup>. Leaf area and leaf area index were recorded on 60, 90 and 120 days after transplanting and at final harvest.

Specific fresh leaf weight at 90 and 120 days after transplanting and at final harvest was obtained by dividing the fresh leaf weight by the leaf area, expressed as mg/cm<sup>2</sup>. The above-ground crop yield, economic yield and cured leaf yield were recorded from each plot and expressed in g/m<sup>2</sup>. The curing in bidi tobacco is done through air-curing and cured leaf yield in bidi tobacco constitutes of four leaf components, viz. the lamina portion, midribs, sand leaves and veins and veinlets.

#### Statistical procedures

Fischer's method of 'Analysis of Variance' was applied for the analysis and interpretation of the data. The level of significance used in 'F' and 't' tests was P=0.05. The values of 'F' and 't' and least significant differences (LSD) were calculated following the method outlined for Randomised Block Design by Panse and Sukhatme (1967).



### 8.3 Results

The fresh biomass of the different trap crops grown during 1993-94 and 1994-95 is presented in Table 2. The sunhemp crop produced more biomass than the other trap crops in both years, whereas the biomass of blackgram and greengram was significantly higher than that of sesamum and soybean in Experiment 1. In Experiment 2, the biomass of greengram was significantly higher than the biomass of redgram, pea and sunflower. In the unweeded control or fallow, no crop was raised.

Table 2. Biomass of different crops at the time of incorporation into the soil at 45 days.

Trap crop	Biomass (t/ha)	
	Experiment 1	Experiment 2
Sunhemp ( <i>Crotalaria juncea</i> L.)	38.3 <sup>c</sup>	31.4 <sup>c</sup>
Greengram ( <i>Vigna radiata</i> L.)	16.6 <sup>b</sup>	16.3 <sup>b</sup>
Redgram ( <i>Cajanus cajan</i> (L.) Millsp.)	-	12.6 <sup>a</sup>
Blackgram ( <i>Vigna mungo</i> L.)	15.3 <sup>b</sup>	-
Pea ( <i>Pisum sativum</i> L.)	-	10.7 <sup>a</sup>
Sunflower ( <i>Helianthus annuus</i> L.)	-	11.0 <sup>a</sup>
Sesamum ( <i>Sesamum indicum</i> L.)	11.1 <sup>a</sup>	-
Soybean ( <i>Glycine max</i> (L.) Merrill.)	10.8 <sup>a</sup>	-
Fallow (control)	-	-
	P	<0.01
	LSD (0.05)	2.6
	CV (%)	9.0

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (P = 0.05).

Table 3. Effect of preceding trap crop on broomrape population in succeeding tobacco at different stages.

Preceding trap crop	Number of broomrapes/m <sup>2</sup>			
	Experiment 1		Experiment 2	
	Early*	Early <sup>+</sup>	Mid <sup>++</sup>	Late <sup>+++</sup>
Sunhemp	0.14 <sup>a</sup>	0.27 <sup>a</sup>	1.10 <sup>a</sup>	1.92 <sup>a</sup>
Greengram	0.14 <sup>a</sup>	0.31 <sup>ab</sup>	1.25 <sup>a</sup>	2.46 <sup>b</sup>
Redgram	-	0.40 <sup>b</sup>	1.58 <sup>b</sup>	2.46 <sup>b</sup>
Blackgram	0.16 <sup>a</sup>	-	-	-
Pea	-	0.60 <sup>c</sup>	1.85 <sup>c</sup>	2.73 <sup>bc</sup>
Sunflower	-	0.63 <sup>c</sup>	1.95 <sup>c</sup>	2.78 <sup>c</sup>
Sesamum	0.21 <sup>a</sup>	-	-	-
Soybean	0.23 <sup>a</sup>	-	-	-
Fallow (Unweeded control)	0.65 <sup>b</sup>	1.01 <sup>d</sup>	3.41 <sup>d</sup>	4.56 <sup>d</sup>
P	<0.05	<0.01	<0.01	<0.01
LSD (0.05)	0.15	0.11	0.25	0.30
CV (%)	46.50	15.90	11.00	8.90

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (P=0.05).

\* 45 days after transplanting tobacco during 1993-94

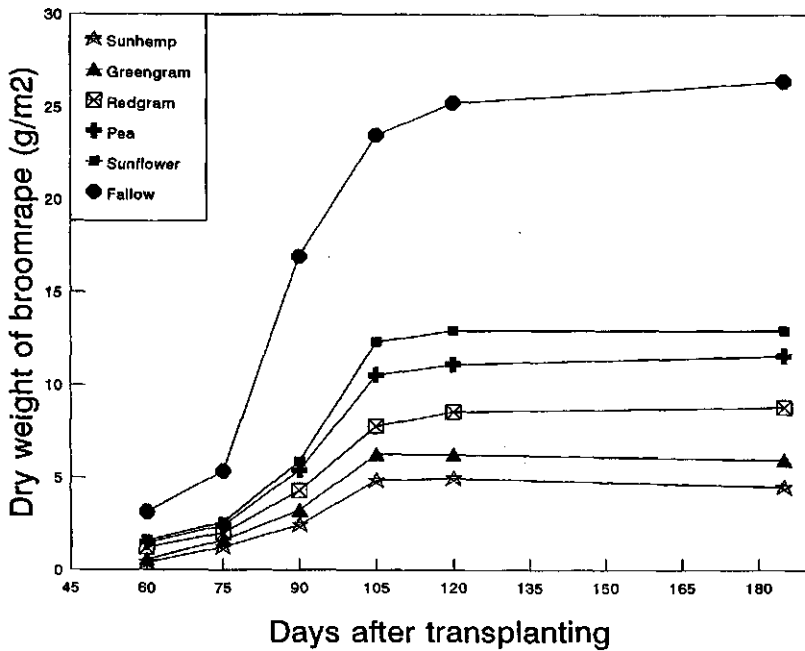
<sup>+</sup> 60 days after transplanting tobacco during 1994-95

<sup>++</sup> 90 days after transplanting tobacco during 1994-95

<sup>+++</sup> Average of values assessed on 105 and 120 days after transplanting tobacco and at harvest during 1994-95

The broomrape population was significantly suppressed by growing trap crops and incorporating their biomass in both experiments (Table 3). In Experiment 1, in which only early infection could be assessed, the suppression was significant but no differences among trap crops could be detected.

In Experiment 2, the broomrape population was significantly suppressed by all trap crops, but their effects were significantly different at all dates of observation. The highest yielding trap crop, sunhemp, showed the best suppressing effect, followed by greengram and redgram. The effects of pea and sunflower were slightly smaller at all dates of observation. Fig. 1 shows the development over time of the dry-matter yield of broomrape. The suppressing effects of the trap crops



LSD (0.05) values for: Trap crops 1.0; Dates 0.6; Crops\*Dates 1.5

Fig. 1. Effects of preceding trap crop on the dry weight of broomrape in succeeding tobacco at different growth stages.

already became apparent early in the growing season. Effects, however, increased during the final phases of the growing season since broomrape yields continued to increase for the fallow treatment, whereas they levelled off for the other treatments.

At 45 days after transplanting, plant height and number of leaves per plant were highest for the treatments where sunhemp or greengram were the preceding trap crops; values for treatments with redgram, pea and sunflower as preceding trap crops were lower but still superior to the fallow treatment (Table 4). Absolute differences in plant height between treatments remained the same during the rest of the growing season. Therefore, final values showed trends similar to the ones on 45 days after transplanting (Table 4). Since the tobacco crop grown on the fallow plots produced fewer leaves after 45 days after transplanting than the other treatments, the treatment effects became more pronounced for the number of leaves when the season progressed (Table 4).

Table 4. Effect of preceding trap crop on the plant height and number of leaves of succeeding tobacco at different growth stages.

Preceding trap crop	Plant height (cm)		Number of leaves	
	45 DAT*	90 DAT**	45 DAT	90 DAT
Sunhemp	51.9 <sup>c</sup>	102.4 <sup>c</sup>	15.7 <sup>c</sup>	19.2 <sup>c</sup>
Greengram	49.8 <sup>c</sup>	100.7 <sup>c</sup>	14.7 <sup>c</sup>	18.3 <sup>c</sup>
Redgram	40.7 <sup>b</sup>	91.1 <sup>b</sup>	12.0 <sup>b</sup>	16.0 <sup>b</sup>
Pea	39.8 <sup>b</sup>	90.2 <sup>b</sup>	11.2 <sup>ab</sup>	15.2 <sup>b</sup>
Sunflower	40.9 <sup>b</sup>	91.5 <sup>b</sup>	11.7 <sup>ab</sup>	16.0 <sup>b</sup>
Fallow (Control)	31.3 <sup>a</sup>	82.5 <sup>a</sup>	10.8 <sup>a</sup>	14.0 <sup>a</sup>
P	<0.01	<0.01	<0.01	<0.001
LSD (0.05)	4.2	3.1	1.1	0.9
CV (%)	6.5	2.7	5.6	3.7

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (P=0.05).

\* Days after transplanting

\*\* Average of values assessed on 90 and 120 days after transplanting and at final harvest

The data pertaining to the above-ground crop yield, the economic yield and the broomrape yield at final harvest as influenced by growing and incorporating different trap crops are presented in Table 5. Highest above-ground crop yields and economic yields were obtained after sunhemp and greengram, followed by redgram; also yields after pea and sunflower were still significantly higher than the control yield.

In contrast, the highest broomrape yield was obtained in the control. Broomrape yields in tobacco preceded by pea or by sunflower were lower than broomrape yields after fallow, but significantly higher than the broomrape yields obtained in tobacco after greengram or sunhemp. The broomrape yield in tobacco after redgram was intermediate. There was a close negative correlation between the above-ground crop yield and the broomrape yield with a linear regression coefficient of -2.8. ( $r^2 = 0.95$ ;  $n = 6$ ).

Values were lower for "infested" tobacco plants than for "non-infested"

tobacco plants but these differences were already established at 60 days after transplanting. The effects of the preceding trap crop on the leaf area per tobacco plant at 60 days after transplanting and at final harvest were significant both in "infested" and "non-infested" tobacco plants (Table 6). The difference between no preceding crop and any preceding crop increased after the first measurement. At 60 days after transplanting and at final harvest, tobacco followed by sunhemp and greengram yielded maximum leaf area in infested and non-infested, whereas leaf area was lowest in the fallow treatment. Values for tobacco preceded by redgram, pea or sunflower crops were intermediate.

Significant differences among the treatments were found with respect to the specific fresh leaf weight or leaf thickness (Table 6) at 90 days after transplanting and at final harvest both in "infested" and "non-infested" plants. Values increased after 90 days after transplanting, especially in "infested" plants and values were (at final harvest) higher for "infested" plants than for "non-infested" plants. Also treatment effects slightly increased over time. Tobacco preceded by sunhemp and greengram gave higher specific fresh leaf weights both in "infested" and "non-infested" plants than the fallow. This was followed by tobacco succeeding redgram, pea or sunflower in "infested" plants. For "non-infested" plants at 90

Table 5. Effect of preceding trap crop on the above-ground crop yield and the economic yield of the succeeding tobacco crop and broomrape yield at harvest.

Preceding trap crop	Tobacco yield (g/m <sup>2</sup> )		Broomrape yield* (g/m <sup>2</sup> )
	Above-ground crop	Economical	
Sunhemp	228.2 <sup>d</sup>	157.0 <sup>d</sup>	4.31 <sup>a</sup>
Greengram	226.8 <sup>d</sup>	155.5 <sup>d</sup>	6.16 <sup>a</sup>
Redgram	209.9 <sup>c</sup>	142.1 <sup>c</sup>	8.37 <sup>b</sup>
Pea	200.3 <sup>b</sup>	116.9 <sup>b</sup>	11.06 <sup>c</sup>
Sunflower	202.9 <sup>b</sup>	116.9 <sup>b</sup>	12.72 <sup>c</sup>
Fallow (Control)	170.4 <sup>a</sup>	90.7 <sup>a</sup>	25.03 <sup>d</sup>
P	<0.01	<0.01	<0.01
LSD (0.05)	6.0	4.7	1.91
CV (%)	2.9	2.9	13.80

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (P=0.05).

\* Average of values assessed on 105 and 120 days after transplanting and at final harvest

Table 6. Effect of preceding crops on leaf parameters of succeeding tobacco at different growth stages.

Preceding trap crop	Leaf area/plant (dm <sup>2</sup> )			
	Infested		Non-infested	
	60 DAT*	Final harvest	60 DAT	Final harvest
Sunhemp	72.2 <sup>d</sup>	92.7 <sup>d</sup>	84.8 <sup>c</sup>	105.3 <sup>d</sup>
Greengram	71.8 <sup>d</sup>	90.2 <sup>d</sup>	84.1 <sup>c</sup>	104.4 <sup>d</sup>
Redgram	64.9 <sup>c</sup>	77.4 <sup>c</sup>	79.7 <sup>b</sup>	92.8 <sup>c</sup>
Pea	64.6 <sup>bc</sup>	71.4 <sup>b</sup>	77.7 <sup>b</sup>	84.8 <sup>b</sup>
Sunflower	62.0 <sup>b</sup>	75.0 <sup>bc</sup>	77.2 <sup>b</sup>	85.9 <sup>b</sup>
Fallow	56.5 <sup>a</sup>	61.1 <sup>a</sup>	68.9 <sup>a</sup>	54.7 <sup>a</sup>
P	<0.01	<0.01	<0.01	<0.01
LSD (0.05)	2.7	4.6	4.7	6.4
CV (%)	2.7	3.9	4.0	4.7

Preceding trap crop	Specific fresh leaf weight (mg/cm <sup>2</sup> )			
	Infested		Non-infested	
	90 DAT	Harvest	90 DAT	Harvest
Sunhemp	73.6 <sup>c</sup>	88.0 <sup>c</sup>	77.4 <sup>c</sup>	80.8 <sup>c</sup>
Greengram	75.7 <sup>c</sup>	88.3 <sup>c</sup>	74.8 <sup>c</sup>	79.5 <sup>c</sup>
Redgram	69.2 <sup>bc</sup>	82.0 <sup>b</sup>	68.9 <sup>b</sup>	73.3 <sup>b</sup>
Pea	70.1 <sup>bc</sup>	77.4 <sup>b</sup>	61.4 <sup>a</sup>	71.3 <sup>b</sup>
Sunflower	66.8 <sup>b</sup>	78.0 <sup>b</sup>	61.3 <sup>a</sup>	68.4 <sup>b</sup>
Fallow	58.4 <sup>a</sup>	67.4 <sup>a</sup>	56.9 <sup>a</sup>	62.3 <sup>a</sup>
P	<0.01	<0.01	<0.01	<0.01
LSD (0.05)	6.7	5.1	4.7	5.0
CV (%)	6.4	4.3	4.7	4.6

\* Different letters indicate significant differences between the treatments based on protected LSD values (P=0.05).

\* Days after transplanting

Table 7. Effect of preceding trap crop on the yields of infested and non-infested plants of succeeding tobacco.

Preceding trap crop	Tobacco yield (g/plant) - "infested" plants					
	60 DAT*			Final harvest**		
	Leaf	Stem	Total	Leaf	Stem	Total
Sunhemp	72.2 <sup>bc</sup>	38.7 <sup>bc</sup>	110.9 <sup>bc</sup>	114.2 <sup>e</sup>	47.5 <sup>e</sup>	161.7 <sup>e</sup>
Greengram	77.0 <sup>c</sup>	44.7 <sup>c</sup>	121.7 <sup>c</sup>	106.1 <sup>d</sup>	43.8 <sup>d</sup>	149.9 <sup>d</sup>
Redgram	65.8 <sup>b</sup>	37.0 <sup>b</sup>	102.8 <sup>b</sup>	94.8 <sup>c</sup>	41.7 <sup>c</sup>	136.5 <sup>c</sup>
Pea	67.3 <sup>b</sup>	34.3 <sup>ab</sup>	101.6 <sup>b</sup>	88.6 <sup>b</sup>	37.9 <sup>b</sup>	126.5 <sup>b</sup>
Sunflower	69.8 <sup>bc</sup>	37.7 <sup>b</sup>	107.5 <sup>b</sup>	91.0 <sup>bc</sup>	39.3 <sup>bc</sup>	130.3 <sup>b</sup>
Fallow	54.0 <sup>a</sup>	28.0 <sup>a</sup>	82.0 <sup>a</sup>	69.3 <sup>a</sup>	31.4 <sup>a</sup>	100.7 <sup>a</sup>
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
LSD (0.05)	7.6	6.8	13.8	4.3	1.9	4.9
CV (%)	7.5	12.2	8.8	3.7	3.9	2.9

Preceding trap crop	Tobacco yield (g/plant) - "non-infested" plants					
	60 DAT*			Final harvest**		
	Leaf	Stem	Total	Leaf	Stem	Total
Sunhemp	90.5 <sup>a</sup>	55.3 <sup>a</sup>	145.8 <sup>a</sup>	126.9 <sup>e</sup>	59.8 <sup>d</sup>	186.7 <sup>d</sup>
Greengram	100.9 <sup>b</sup>	62.9 <sup>b</sup>	163.8 <sup>b</sup>	122.4 <sup>d</sup>	59.7 <sup>d</sup>	182.1 <sup>d</sup>
Redgram	89.7 <sup>a</sup>	54.3 <sup>a</sup>	144.0 <sup>a</sup>	109.4 <sup>c</sup>	54.9 <sup>c</sup>	164.3 <sup>c</sup>
Pea	93.8 <sup>ab</sup>	54.8 <sup>a</sup>	148.6 <sup>a</sup>	102.6 <sup>b</sup>	52.7 <sup>b</sup>	155.3 <sup>b</sup>
Sunflower	91.7 <sup>a</sup>	53.0 <sup>a</sup>	144.7 <sup>a</sup>	105.8 <sup>b</sup>	52.9 <sup>bc</sup>	158.7 <sup>b</sup>
Fallow	85.8 <sup>a</sup>	51.3 <sup>a</sup>	137.1 <sup>a</sup>	95.7 <sup>a</sup>	48.8 <sup>a</sup>	144.5 <sup>a</sup>
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
LSD (0.05)	8.3	7.3	13.8	3.4	2.2	4.7
CV (%)	6.0	8.8	3.2	2.5	3.3	2.3

<sup>a</sup> Different letters indicate significant differences between the treatments based on protected LSD values (0.05).

\* Days after transplanting

\*\* Average of 120 days after transplanting and at final harvest

days after transplanting, the effects of pea or sunflower were not significant. At final harvest, tobacco preceded by redgram, pea and sunflower in "infested" and "non-infested" recorded moderately but significantly higher specific fresh leaf weights compared to the fallow treatments; their effects were similar.

The effects of the preceding trap crop on the dry weight of leaf, dry weight of stem and the total dry matter both in "infested" and "non-infested" plants are presented in Table 7. Significant treatment effects were found in dry weight of leaf, stem and the total dry matter at 60 days after transplanting both in "infested" and "non-infested" plants. Effects were much larger in "infested" plants than in "non-infested" plants and increased over time. Trends were very similar for leaf and stem fractions. Tobacco preceded by any crop ultimately produced significantly higher quantities of dry matter than the unweeded control, and the effects increased in the order pea, sunflower, redgram, greengram and sunhemp in both "infested" and "non-infested" plants. Both in "infested" and in "non-infested" plants, however, the differences among treatments changed over time, mainly caused by the fact that tobacco after sunhemp, the trap crop with the largest yield, continued to produce rapidly until the final harvest.

#### 8.4 Discussion

The objective of this research was to find a trap crop for the kharif season, which not only suitably precedes the tobacco in the rabi season and traps the parasite, but also enhances the tobacco yield through the green manuring effects. The superiority of a trap crop over other crops largely depends on the root densities of the crop, the root area duration, the exudate production and the quality and the concentration of the stimulant in the root exudates produced by the roots of the trap crops, or simply on the amount of green manure incorporated in the soil. The water use of the trap crop, however, should also be taken into account.

Meteorological data for the experimental seasons, reveal that the rainfall pattern and the rainfall amounts were different. Rainfall during 1993-94 was about 290 mm more than the normal rainfall of 720 mm. Especially during October 1993, the rainfall was extremely heavy. This most likely caused the snubbing of broomrape during 1993-94. During 1994-95, the rainfall during July was 375 mm, much more than the normal rainfall of 163 mm. This excessive rainfall may have enhanced secondary dormancy of *Orobanche cernua* resulting in delayed infection in the 1994-95 experiment (Ter Borg, 1986). Because of this ample rainfall, it is unlikely that the tobacco crop suffers from water stress, caused by the transpiration of the trap crop.

The incidence of *Orobanche cernua* in Experiment 1 at 45 days after transplanting tobacco is appropriate because in the black soils of the Nipani area,



the emergence of the parasite could be seen 38 - 40 days after transplanting tobacco. Krishnamurthy et al. (1977) reported that *Orobancha cernua* takes 15 - 20 days for the emergence above the ground after it has infected the roots of tobacco. Maximum *Orobancha cernua* population was observed late in the growing season. This is mainly due to the time required to obtain maximum volume and mass of the roots of the tobacco crop, resulting in more soil explored, more root exudates produced, and therefore more *Orobancha* seeds stimulated to germinate and attach.

Significantly higher broomrape yields were obtained after fallow than for treatments with a preceding crop. Krishnamurthy and Umamaheshwara Rao (1976) also observed that alternate cropping helps to control *Orobancha cernua* and found a maximum number of broomrape spikes in tobacco after fallow. All trap crops reduced the number of broomrape spikes and the dry weight of broomrape. The lowest broomrape yield was obtained in tobacco preceded by sunhemp. The biomass of the sunhemp trap crop was significantly higher than the biomass of all the other trap crops. The lower broomrape yields after trap crops are probably due to suicidal germination of a portion of the seed reserve which was ready to infect the tobacco crop. This resulted in fewer broomrapes per unit area but also lower yields per broomrape spike were found. These results confirm the laboratory studies of Krishnamurthy and Chandwani (1975) and Dhanapal and Struik (1996) and the field study of Hosmani (1985). Even with similar yields of trap crops, the number and yield of broomrapes could be different. This may be due to the higher production of root exudate, a higher stimulant concentration, a higher root:shoot ratio or a larger root surface.

It could also be hypothesised that addition of organic matter by incorporating trap crops into the soil encourages the depletion of viable seed in the soil and/or the growth of the host plants. Suicidal germination of the parasitic seeds triggered by growing trap crops reduced the weed seed inoculum in the soil and also, the growth of the host plants was hastened due to the green manuring effect of trap crops. In the latter case, such an effect would be reflected both in "infested" and "non-infested" plants. However, the difference between these two categories of tobacco plants was smaller for the trap crop treatments than for the fallow treatment and the maximum effect of the crop compared to the fallow was larger for "non-infested" plants than for "infested" plants. Probably the trap crop effect was a combination of green manuring and trapping. There was no systematic difference between the leguminous trap crops and the non-leguminous ones.

Krishnamurthy et al. (1977) estimated the loss in tobacco yield to an extent of 24 - 52% in Andhra Pradesh depending on the time and intensity of infestation of *Orobancha cernua*. Murthy and Nagarajan (1986) observed that broomrape infestation reduced the plant height by 52%, the number of leaves by 34%, the dry

weight of the stem by 34% and the dry weight of the roots by 54% in tobacco. In the present investigation, a reduction in economic yield of tobacco by 42% with a broomrape population of 58% at harvest was observed. Further, *Orobanche cernua* infestation reduced the plant height by 19%, the number of leaves by 32%, the dry weight of leaves by 39%, the dry weight of stem by 34% and the total dry matter by 38% in the infested plants at final harvest. We also observed that the parasite reduced the leaf area of tobacco by 34% and specific fresh leaf weight by 23% in infested plants at final harvest. At 90 days after transplanting tobacco sunhemp and greengram trap crops reduced *Orobanche cernua* population by 68%.

The broomrapes reduced the yields of their hosts much more than they invested in their own above-ground parts. It was suggested that broomrape dry weight does not compensate for loss of host dry weight, at least partly because the respiration of early structures of *Orobanche* is so huge (Ter Borg et al., 1994; see also De la Harpe et al., 1981 and Riopel and Timko, 1995). Also the host tissue can show increased respiration after an infection by *Orobanche* (Singh and Singh, 1971). For another part this discrepancy between yield loss and production of the parasite is caused by the effect of broomrape on the architecture of the host and on the functioning of the host (Graves, 1995).

Due to suicidal germination of *Orobanche cernua* seeds triggered by trap crops, *Orobanche* seed reserve in the soil may be reduced. If growing of trap crops is frequent this effect may be beneficial in the long run. The immediate effect is that fewer seeds are able to germinate in the next tobacco crop, thereby appreciably increasing yields. Most promising crops are sunhemp and greengram. Sunhemp, being a succulent crop, is rapidly decomposable and high yielding; it had a large green manuring effect. Greengram is a leguminous crop; it enriches the nitrogen uptake of the succeeding tobacco. Redgram, showing retarded growth until 90 days after sowing, pea and sunflower proved less effective trap crops. Bouhatous and Jacquard (1994) suggested that crop mixtures can be more efficient as trap or catch crops than monocultures.

Thus, sunhemp and greengram are promising trap crops to be included in a cropping system containing bidi tobacco in the Nipani tobacco area where tobacco is grown in a long growing season during the late kharif/rabi seasons.

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## Chapter 9

## Chapter 9

### GENERAL DISCUSSION

In this Chapter, the results of Chapters 3-8 will be linked and discussed in a broader context, and translated into an integrated approach of *Orobanche* control in the tobacco growing area of Nipani, India.

#### *The bidi tobacco production system in Nipani and its relation to broomrape infestation*

Bidi tobacco is one of the non-Virginia tobaccos cultivated in India; it has been grown in the Nipani area of the Belgaum district (Karnataka) for the past 40 years. Bidi tobacco is being grown in this area in medium black soils with a pH of 8.1 - 8.5. The tobacco crop is raised during late spring and winter seasons, i.e. from August till March. Nipani experiences dry hot summers, humid and cool monsoons and mild winters, with an average annual rainfall of 720 mm. Continuous cropping of tobacco under rainfed conditions is the normal practice in the Nipani area. This has resulted in a very high level of broomrape infestation. The parasite is not infesting the tobacco nurseries but heavily infests tobacco briefly after transplantation into the field.

In India *Orobanche cernua* appeared as early as 1945 and again in 1968 in epidemic quantities, destroying the entire tobacco crop. The parasite debilitates the tobacco plants severely, resulting in stunted growth and reduced yields. The parasite is indigenous; the severity of its attack depends on rainfall received during the crop season: rainfall stimulates the development of the parasite as well the growth of the crop. Tobacco yields are further reduced because of the fact that the parasite also hosts many tobacco pests like cutworms, leaf eating caterpillars, capsule borers and aphids. The severity of the broomrape problem in the Nipani area is increasing because of continuous cropping of the tobacco crop. In the Nipani area hand weeding is the only present practice in controlling the parasite, which is extremely laborious or virtually impossible in heavily infested tobacco fields. The present study aimed at developing methods of control of *Orobanche cernua* in the local situation.

#### *Field observations on the damage by Orobanche cernua*

Tobacco growth is greatly impeded by *Orobanche cernua* infestation. An overview

of the effects is presented in Table 1. The effect on plant height was relatively small (about 19%) compared to the effect on yield, especially of the harvestable product, viz. the leaves. Crop losses are not "compensated" by dry-matter yields of broomrape spikes. The harvest index and the quality of the tobacco leaf are also affected by the infestation of *Orobanche cernua*.

Tobacco height was more reduced when there were more broomrape spikes per tobacco plant. It was reduced by 22 - 27% with 11 - 15 spikes per tobacco plant and by 15 - 17% with 6 - 10 spikes per tobacco plant compared to the height of plants with only 0 - 5 broomrape spikes per tobacco plant at 110 - 185 days after transplanting. There was a close negative linear relationship ( $r^2=0.588$ ;  $n=50$ ) between the number of spikes per plant and the plant height at final harvest. The negative linear relationship between number of spikes per plant and leaf dry weight of tobacco was even more significant ( $r^2=0.825$ ;  $n=50$ ). The effects of the number of spikes per tobacco plant on the average spike height was not significant, but there was a highly significant ( $r^2=0.581$ ;  $n=50$ ), close negative exponential relationship between number of broomrape spikes per tobacco plant and their average dry weight.

Table 1. Overview of effects of broomrape infection on performance of tobacco in Nipani, India. For definition of "infested" and "non-infested" plants see Chapter 5.

	% reduction of	
	"infested" plants	"non-infested plants"
<u>Growth of tobacco</u>		
Plant height	19	.
No. of leaves	32	.
Leaf area	34	.
Yields (dry weight)		
Leaves	39	25
Stem	34	17
Total	38	23
<u>Other</u>		
Specific fresh leaf weight	23	20

Relating biological dry-matter yield of tobacco c.q. loss of dry weight to broomrape dry weight is hardly possible in this tobacco production system,

because the tobacco growth is strongly affected by cultural practices:

- By 'topping', the apical meristems are removed to prevent flowering and seed production.
- All axillary buds and suckers are removed, to prevent formation of branches and shoots.

It might be worthwhile to try to quantify the effect of broomrape infestation on production of those buds and suckers. In case of an appreciable effect, this might influence the amount of labour required in this activity, and thus might have economic consequences.

### *The three control strategies investigated*

Broomrapes can be controlled in the seed and germination phase, in the hypogeal phase, when seedlings and young parasites are establishing on their hosts, and after emergence.

The different strategies tested are evaluated in Table 2, as far as adequate information was obtained. Plant oils and hand weeding are laborious but also more successful in reducing the population of broomrape. However, their effects on tobacco yield were not evaluated. Growing of a trap crop and chemical treatments showed similar effects on broomrape spike numbers and yield. The different strategies tested are further discussed below.

Table 2. Summary of the effectiveness of different approaches in the control of *Orobancha cernua* in tobacco in Nipani, India.

Approach	Obtained reduction in broomrape numbers (%)	Increased yield of tobacco (%)	Disadvantage
Chemical induction of germination	Unknown	Unknown	currently no chemical control
Trap crops	68	45	loss of resources
Glyphosate/imazaquin	80	43	crop damage
MH	75	48	crop damage
Plant oils	90-100	Unknown	laborious
Hand weeding	90-100	Unknown	laborious



### *Control by inducing germination*

In practice, seeds of *Orobancha cernua* germinate only under the influence of root exudates of host or non-host plants. If such seeds are stimulated to germinate by using chemicals or root exudates of trap crops, germination may occur, and the seedlings of such germinated seeds die, due to the lack of suitable hosts. This phenomenon is known as suicidal germination.

The effectivity of a number of chemicals as germination stimulants was tested, and compared with that of GR24. These chemicals included GA (gibberellic acid), ethephon, NAA (naphthalene acetic acid), BA (benzyl adenine) and pyridoxine. All of them appeared to be rather effective. Although the germination percentages attained with them were significantly lower than the values reached with GR24, the difference was relatively small. With respect to GA earlier authors sometimes found a similar stimulating effect when applying GA during germination. Mostly, however, it was only found to be effective during the conditioning phase (cf. Parker and Riches, 1993, for a review). Since the data published concern a range of various broomrapes, a comparative study, including *O. cernua* and other *Orobancha* species, preferably collected at various sites, is required for better understanding of the germination process.

Generally the strigol analogues like GR24 are much more effective, and might be applied in the soil to stimulate germination. However, until now they are only in the very first stage of development as a practical herbicide. Moreover, the seed bank of the parasite is huge in the entire tilth and seeds can only be induced to germinate over short distances. Therefore, large quantities of chemicals are needed to obtain a significant effect, similar to the quantities used for soil fumigation against nematodes.

The effects of various possible trap crops were tested; some of them, greengram (*Vigna radiata*) and sunhemp (*Crotalaria juncea*) in particular, appeared to have a definite positive influence on crop yield. This could be due to the fact that trap crops may stimulate the germination of the parasitic seeds. Data of laboratory studies on the stimulatory capacities of the root exudates and data of field experiments on the reduction in broomrape spike density were consistent (Table 3), which suggests that the positive effects of growing certain crops before growing tobacco can at least partly be attributed to exhaustion of the (non-dormant) seed bank due to suicidal germination brought about by the root exudates of the trap crops. In such case, trap crops with high root density are preferable to induce maximum seed germination.

The positive interaction between the high concentration of GR24 and the root exudates of some crops (Chapter 4) deserves further attention.

Trap crops, in particular leguminous species such as greengram, in a tobacco

cropping system may also be beneficial because of their green manuring effect and their role in nitrogen fixation. To reach a maximal effectivity, yield of the trap crop should be maximal.

Use of trap crops requires adequate water and a long growing season. In our experiments tobacco still appeared to produce good yields in the period left after growing a trap crop. In view of the strong reduction of broomrape numbers (Table 2) brought about by the crops studied, a system of a trap crop followed by a short fallow, then tobacco crop, seems to offer good opportunities in the Nipani area.

Table 3. Relative effects of trap crops on germination (laboratory experiments) or control in successive tobacco crop (field experiments). See Chapters 4 and 8 for details.

0 = no effect; 1 = small effect; 2 = medium effect; 3 = large effect; 4 = very large effect  
 . = not observed or included.

	Laboratory experiments*				Field experiments**		
	Exp. 3	Exp. 6	Exp. 7	Average	Exp. 1	Exp. 2	Average
Sunhemp	4	3	.	4	3	3	3
Blackgram	3	3	3	3	3	.	3
Greengram	3	3	3	3	3	2	3
Sesamum	3	3	.	3	2	.	2
Sunflower	2	3	2	2	.	1	1
Redgram	1	1	1	1	.	2	2
Soybean	1	1	1	1	2	.	2
Pea	1	1	.	1	.	1	1

\* Numbers indicate experiments in Chapter 4

\*\* Numbers indicate experiments in Chapter 8

*Control by herbicides after transplanting the tobacco crop*

Herbicides kill the parasite effectively thus preventing the seed set. Only selective herbicides, either systemic or non-systemic ones, can be employed in the tobacco crop to reduce the spikes of *Orobancha cernua*. Systemic herbicides like glyphosate when applied on the foliage of the tobacco crop, enter the plant system and kill the broomrape attachments, while contact herbicides simply kill the parasite by direct exposure. The herbicides glyphosate and imazaquin at 0.50 and 0.01 kg a.i./ha respectively reduced the number and dry weight of broomrape spikes. The same held for MH (maleic hydrazide) at 0.25 kg a.i./ha. These treatments had no adverse effects on the host plant. At higher doses broomrape numbers were reduced somewhat more, but the herbicides then were toxic to tobacco; this held for MH

(maleic hydrazide) in particular: 0.50 and 0.75 kg a.i. MH/ha reduced the broomrape spikes greatly but was toxic to tobacco. Chemicals at their higher concentrations reduced the tobacco yields both in "infested" and "non-infested" tobacco plants even though broomrape control was satisfactory. No chemical treatment could reduce the broomrape effects to such an extent that tobacco yields were obtained similar to those under the hand weeding treatment, or in a situation where no infestation of broomrape occurred.

Unless the crop is fully resistant to the herbicide it is impossible to get proper control of broomrape through treatment with a systemic herbicide without crop damage. Designing a herbicide with a large selectivity based on physiological mechanisms would be a better approach than testing numerous herbicides in different concentrations and at different dates of application. In other crops, such as faba bean and sunflower, glyphosate resistance is present.

In view of the latest literature reports on use of these and other new herbicides (see e.g. overview by Eplee and Norris, 1995) the concentrations tested in the research described are relatively high, especially in the experiment with maleic hydrazide. It is possible that similar control of broomrape can also be obtained by lower concentrations, whereas the crop damage might be smaller than observed in the current experiments. Further research to optimize the concentrations is required. Also the timing of the application(s) of the herbicides can be optimized. Although the effects of time of application were usually relatively small, such effects found for glyphosate (late application better) and imazaquin (early application better) might be relevant.

#### *Control by plant oils*

Natural plant oils can be used to kill the young broomrape spikes, thereby preventing the seed set. These plant oils do not cause environmental problems and can be used effectively. Because of the risk of re-growth of stems, killing the entire spike is desirable.

Some of the oils tested appeared to be effective. In India, labour is not a problem; these plant oils are relatively cheap and non-toxic to host plants and the environment and can be used to control broomrape, but their application technology is to be worked out. Neem oil, coconut oil, sunflower oil were most effective in killing the bud part of the parasite whereas neem and mustard oils were ineffective in killing the stem. To identify the mechanisms of the effects of the oils and their most effective components, more research is required.

### *Integrated control*

Integrated control includes physical, chemical, cultural and biological methods. In the present investigations, attempts were made to control the parasite by cultural and chemical approaches. Krishnamurthy (1994) has suggested:

- to use trap crops or catch crops together with hand weeding using spear and minispear tools, or
- to apply deep summer ploughing and crop rotation together with hand weeding using the spear or minispear tools.

In our investigation, greengram and sunhemp appeared promising trap crops for the Nipani area. Glyphosate at 0.50 kg a.i./ha and imazaquin at 0.01 kg a.i./ha were found rather safe to tobacco and effective in controlling the broomrape spikes. Hand weeding, however, is still the best control treatment.

Therefore, I suggest the following package to obtain higher tobacco yields and minimize the *Orobanche cernua* population in the soil for the Nipani tobacco area and areas of similar conditions.

1. Grow trap crops (sunhemp or greengram) in the early spring and incorporate *in situ* 45 days after sowing.
2. After 15-20 days transplant tobacco.
3. Take up general weeding within 45 days after transplanting.
4. Apply glyphosate at 60 DAT at 0.50 kg a.i./ha (or less).
5. Remove the remaining few broomrape spikes by hand or apply plant oils to prevent seed formation.

More research on the trap crop approach in combination with the use of chemicals is necessary to optimise the technology.

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## SUMMARY

### *The tobacco crop*

Tobacco (*Nicotiana tabacum* L.) is an important commercial crop of India. India is the third largest tobacco producing country in the world. Non-Virginia tobacco such as bidi tobacco constitutes about 65% of the total area and about 73% of the total tobacco production in the country. The present study was conducted in Karnataka State, where bidi tobacco production is strongly affected by broomrape infestation.

### *The broomrape weed*

Broomrapes (*Orobancha* spp.) are phanerogamic, a-chlorophyllous holoparasitic plants which parasitize many economically important crops. The tiny seeds germinate only under the influence of root exudates up to a distance of a few millimetres from the root of the host plant or certain non-host plants. After attachment to their host, they establish connections with the host vascular system, draw water, nutrients and metabolites through haustoria that penetrate the root tissue, and disrupt the growth and development of host plants to such an extent that yield losses are a multiple of the (above-ground) yield of the weed.

Broomrapes parasitize a limited number of plant species; they include wild plants and crops. *Orobancha cernua* Loefl. on Solanaceous crops and *Orobancha aegyptiaca* Pers. on Cruciferous crops are the major species found in India. *Orobancha cernua* appears in all tobacco growing areas in India and has a devastating effect on the crop. Yield loss ranges from 30 - 70%, depending on the time and severity of the infestation of the parasite. In some regions the problem even tends to increase.

Various control methods such as crop rotation, deep ploughing, use of fertilizers, growing of trap crops, solarization, use of resistant varieties, and biological and chemical techniques have been evaluated for broomrape control. However, currently no single method proved to be effective and economically feasible to control the parasite. Control may focus on different stages of development of the parasite viz. the seed phase, the germination phase and the parasitic/reproductive phase. The practice of combining physical, chemical, cultural and biological methods in controlling the parasite offers the key to success. Henceforth, it is generally agreed that integrated control offers the best perspectives in its management.

The research presented in this thesis is focussed on the germination biology of *Orobancha cernua* to study the potential of control through suicidal germination, on identifying suitable trap crops, testing the effectiveness of post-emergence,

systemic herbicides and testing the efficacy of natural plant oils. Results should contribute to the development of a technology which integrates cultural and chemical approaches in managing *Orobanche cernua* in the bidi tobacco crop.

#### *Effects of broomrape on tobacco*

*Orobanche cernua* parasitizes all types of tobacco alike, and causes reduced crop growth, wilting and ribbed appearance of leaves, and chlorosis. In the tobacco growing areas of Nipani, Karnataka State, the parasite infects the tobacco roots in the second week after transplanting tobacco and completes its below-ground development until the fifth week. It emerges above-ground from the sixth week onwards. The life cycle of the parasite is completed in about 3 months after transplanting tobacco. The major part of the broomrape spikes occurred on tobacco plants carrying 4 - 9 spikes per plant. The tobacco crop grew exponentially till 60 days and thereafter, linearly in the period 60 - 140 days after transplanting. Later on, the growth became truncated. Broomrape height levelled off after 140 days. The broomrape effect was mainly through a reduction of growth rate and not by shortening the growth period. Tobacco height and dry weight of tobacco leaves were more reduced when there were more broomrape spikes per tobacco plant. The number of broomrape spikes per tobacco plant had no significant effect on the height of the broomrape spikes themselves, but the curvilinear, negative relation between number of spikes and average spike dry weight per host was highly significant.

#### *Effect of chemical and natural stimulants on the germination of Orobanche cernua seeds*

The germination phase of *Orobanche cernua* is a critical period during which control may be effective. The effect of chemical and natural stimulants or a combination of the two on germination of the parasitic seeds was therefore studied in the laboratory (Chapter 4); GR24 at 1.0 and 0.1 ppm was the standard to assess potential germination. Of the other chemicals, GA, NAA, BA and pyridoxine also considerably increased germination. Stimulatory capacities of root exudates of a number of possible trap crops were tested. Exudates of sunhemp (*Crotalaria juncea*) and greengram (*Vigna radiata*) strongly induced broomrape seed germination, whereas blackgram (*Vigna mungo*), sunflower (*Helianthus annuus*) and sesamum (*Sesamum indicum*) were also rather effective. Redgram (*Cajanus cajan*), pea (*Pisum sativum*) and soybean (*Glycine max*) had no relevant effect. Whereas these host species differed greatly in their ability to induce germination, no variation was observed among cultivars within a host species. The effects of chemicals and trap crops interacted. A positive interaction was even observed between the effects of root exudates and GR24 at 1.0 ppm. Apparently

germination is not maximal with GR24 alone, but can be further stimulated by additional effects of a host.

These results fairly well correlated with field experiments on broomrape control using trap crops (Chapter 8). The investigations revealed that the trap crops, especially sunhemp and greengram, significantly reduced the numbers of broomrape spikes, and resulted in higher tobacco yields. Redgram, sunflower and pea trap crops had similar effects but were less effective. Highest broomrape density of 4.6 spikes/m<sup>2</sup> was recorded in the fallow (control treatment); the trap crop treatments showed spike densities of 1.9 - 2.8/m<sup>2</sup> at harvest. A decrease of 79% in the dry weight of broomrapes in sunhemp and greengram trap crop treatments was noticed compared to the fallow treatment (control). The superiority of one trap crop over others may depend on the root density, the root area duration, the exudate production, or simply on the quantity of green manure incorporated into the soil. Therefore, growing trap crops could increase yield by depletion of viable seeds in the soil through suicidal germination or by the green manuring effect.

#### *Role of herbicides in the post-emergence control of *Orobanche cernua**

In field experiments, the effect of chemicals on broomrape control were studied in two steps (Chapter 5). In a primary screening trial, 15 herbicides were evaluated for their selectivity in tobacco and their efficiency in controlling the parasite. This study helped to narrow down the choice of chemicals for further testing. In the second trial, glyphosate, imazaquin, imazethapyr and EPTC, found selective in the primary screening trial, were tested, comparing two dates of application and two concentrations. Best results were found with glyphosate at 0.50 kg a.i./ha applied at 60 days after transplanting and imazaquin at 0.01 kg a.i./ha applied at 30 days after transplanting; they reduced the broomrape population by 77 - 80% and increased the tobacco yields (leaf dry weight) by 43% as compared to the control. Imazethapyr and EPTC proved to be less effective. All chemicals were toxic to host plants at their higher concentrations. Glyphosate at 0.50 kg a.i./ha applied at 30 days after transplanting showed some crop injury at the tip of the plant but later the plants recovered. The dry weight of broomrapes at harvest was 18.6 g/m<sup>2</sup> in the unweeded control treatment; in the chemical treatments the range was 2.8 - 10.1 g/m<sup>2</sup>. Hand weeding treatment topped over all chemical treatments. Chemicals at their lower concentrations produced higher yields than the unweeded control treatment.

In another field experiment it was shown that maleic hydrazide, a systemic foliar herbicide, inhibited the infection of the parasite at 0.25 - 0.75 kg a.i./ha applied at 30 or 40 days after transplanting tobacco. Higher tobacco yields were obtained with 0.25 kg a.i./ha maleic hydrazide, which was on par with the hand

weeding treatment. Higher concentrations of maleic hydrazide were toxic to tobacco crop. The concentration applied was critical but the application time was not crucial (Chapter 6). The results indicate that effective control of broomrape in bidi tobacco without adverse effects on crop growth and yield is difficult to achieve with a single foliar application of maleic hydrazide.

#### *Effect of plants oils on young broomrape spikes*

In an effort to identify natural plant oils for post-emergence control of *Orobanche cernua* in tobacco crop several plant oils were tested (Chapter 7). Plant oil at a rate of 0.09 ml/broomrape spike was applied directly on the tip of the spike using a dropper. Observations were recorded on the number of days taken to bring about the mortality of the broomrape bud and the stem part of the parasite. Also the phytotoxicity of plant oils on tobacco plant was observed. Swabbing natural plant oils killed the bud and stem part of the parasite by a toxic effect. Neem, coconut and sunflower oils showed a quick knock-down effect, whereas castor, niger and mustard oils took more days. All plant oils tested killed the emerged spikes completely, except for neem and mustard oils which killed the bud, but not the stem part of the parasite. No phytotoxic effects on the tobacco were found.

#### *Conclusions*

Tobacco growth is reduced by *Orobanche cernua*. The effect of broomrape was mainly through a reduction of growth rate and not by shortening the growth period. The reduction in the plant height of tobacco depended upon number of broomrape spikes per tobacco plant. In the future, part of the problem may be controlled by chemicals stimulating germination in the absence of a host, by growing a trap crop, or a combination of the two. In the laboratory, the positive effects of trap crops were observed even when there was already a strong stimulation by chemicals.

Glyphosate at 0.50 kg a.i./ha applied at 60 days after transplanting and imazaquin at 0.01 kg a.i./ha applied at 30 days after transplanting were efficient chemicals in controlling *Orobanche cernua* in a tobacco crop. With a single foliar application of maleic hydrazide, it is difficult to achieve a control of the broomrape without any adverse effects on the crop. Higher concentrations are toxic to the tobacco plant. Coconut oil and sunflower oil were effective in killing the parasite without damage to tobacco. However, each spike must be treated individually which makes control laborious.

No single method is effective in controlling the parasite. Therefore, an integrated control strategy offers the best perspectives to control broomrapes. A package to be tested should include growing a trap crop followed by a herbicide treatment in the tobacco crop; later in the growing season additional hand weeding



or treatment with plant oils can be carried out to remove the last spikes left, to prevent production of new seeds and exhaust the soil seed bank.

## SAMENVATTING

### Tabak

Tabak (*Nicotiana tabacum* L.) is een belangrijk gewas in India; dit land is de derde tabaksproducent van de wereld. Behalve Virginia tabak, bestemd voor de produktie van sigaretten, worden op grote schaal ook vele andere variëteiten zoals bidi tabak verbouwd. Het hier gepresenteerde onderzoek werd uitgevoerd in Nipani in de staat Karnataka, waar de teelt van bidi tabak de laatste decennia in toenemende mate te lijden heeft van aantasting door bremraap.

### Bremraap

Bremrapen (*Orobanche* spp.) zijn phanerogame, chlorofylloze, parasitaire planten; enkele soorten uit dit geslacht kunnen optreden als onkruiden in belangrijke gewassen en veroorzaken daar vaak grote schade. Er bestaat een nauwe relatie tussen de waardplant en de parasiet, doordat de kieming van de zeer kleine zaden van deze onkruiden wordt geïnitieerd door wortellexsudaten van de waardplanten, en ook wel door andere soorten die niet als waard kunnen optreden maar wel de kieming op gang kunnen brengen. De kieming wordt alleen gestimuleerd als het zaad zich op korte afstand van de wortels bevindt. Na kieming vindt hechting plaats aan de wortels van de waard. Via een haustorium wordt een verbinding tot stand gebracht met diens vaatbundelstelsel. Daarmee kan de parasiet water, nutriënten en assimilaten onttrekken aan de gastheer. Deze wordt hierdoor danig in zijn groei geremd. Opbrengstverliezen vormen vaak een veelvoud van het gewicht van de bremraap.

Er zijn verschillende beheersingsstrategieën bekend, zoals vruchtwisseling, diepploegen, bemesting, teelt van lok- of vanggewassen, solarisatie, gebruik van resistente rassen, en inzet van biologische of chemische technieken. Tot nu toe is geen van deze methoden afzonderlijk afdoende of economisch haalbaar gebleken. Een geïntegreerde aanpak met fysische, chemische, agronomische en biologische technieken, gebruik makend van de zwakheden van het onkruid tijdens verschillende ontwikkelingsstadia, heeft nog de beste kans van slagen.

In India is *Orobanche cernua* Loefl. berucht. Deze soort tast vooral Solanaceae aan, waaronder tabak, en vormt een plaag in alle belangrijke teeltgebieden van dit gewas in India. Opbrengstverliezen van 25 tot 50% zijn gewoon, maar ook verliezen tot 70% komen voor. Het probleem lijkt bovendien in sommige gebieden toe te nemen. Ook hier lijkt alleen een combinatie van methoden tot een oplossing te kunnen leiden.

Dit proefschrift richt zich op de kiemingsbiologie van *O. cernua*, om de mogelijkheden tot het induceren van "zelfmoord-kieming" na te gaan, op het

identificeren van geschikte vanggewassen, het testen van enkele systemische en niet-systemische herbiciden, en het testen van plantaardige oliën. Samen moeten deze technieken bijdragen aan een geïntegreerde aanpak van het probleem in de tabaksteelt.

#### *De levenscyclus van bremraap en tabak, en hun interactie*

Tabak wordt voorgekweekt en vervolgens in het veld uitgeplant. De eerste 60 dagen na planten vertoont het gewas een exponentiële lengtegroei. De groei is lineair in de periode 60 - 100 dagen na planten. De groei vlakt vervolgens af, maar het gewas blijft nog tot ongeveer 185 dagen na planten te velde.

*O. cernua* komt voor op alle tabaksvariëteiten en veroorzaakt een groeiremming, verwelking, geribbeld blad en chlorose. In de tabaksteelt in de omgeving van Nipani wordt het gewas in de tweede week na planten door de parasiet geïnfecteerd. De eerste weken blijft de parasiet ondergronds. Ongeveer zes weken na het planten komen de eerste scheuten boven. De levenscyclus van de bremraap wordt in ongeveer 100 dagen voltooid. Nieuwe scheuten verschijnen zolang de waardplant levende wortels heeft. De meeste tabaksplanten hebben 4 - 9 scheuten, met uitschieters tot 15 bremrapen per plant.

De bremraap zorgt voor opbrengstreductie bij tabak door een afname van de groeisnelheid; de groeiduur wordt niet beïnvloed. De gewasgroei wordt des te sterker belemmerd naarmate er meer scheuten per plant ontwikkelen. De onderlinge competitie tussen bremraapscheuten aan één tabaksplant resulteert niet in een geringere gemiddelde planthoogte, maar het gemiddelde drooggewicht van de bovengrondse delen neemt exponentieel af met een toename van hun aantal.

#### *Effecten van chemische en natuurlijke stimulantia op de kieming*

De kiemingsfase van bremraap is een kritieke periode, waarin bestrijding effectief kan zijn. Daarom werden de effecten van chemische en/of natuurlijke stimulantia onder laboratorium-omstandigheden onderzocht (Hoofdstuk 4), waarbij het strigol analogon GR24 werd gebruikt als standaard om de potentiële kieming vast te stellen. Van de geteste stoffen bleken  $GA_3$ , NAA, BA en pyridoxine een stimulerende werking te hebben. Ook de wortel-exsudaten van enkele vanggewassen - *Crotalaria juncea* L., *Vigna radiata* L. en in mindere mate die van *Vigna mungo* L., *Helianthus annuus* L. en *Sesamum indicum* L. - stimuleerden de kieming van bremraap zaden. Gelijktijdig toedienen van 1.0 ppm GR24 en wortel-exsudaten leidde tot een extra verhoging. Terwijl de verschillende soorten vanggewassen onderling grote verschillen vertoonden, werden binnen soorten geen rasverschillen aangetroffen.

Vergelijkbare effecten werden waargenomen in een veldproef, waarin de effecten van de teelt van vanggewassen op de bremraapaantasting van een nateelt van tabak werden onderzocht (Hoofdstuk 8). Ook hier onderdrukten vooral *C. juncea* en *V. radiata* de bremraap-aantasting, en werd een hogere tabaksopbrengst verkregen. Bij de eind oogst was de bremraapdichtheid 4,6 planten per m<sup>2</sup> in de veldjes die braak hadden gelegen voordat de tabak geplant werd. In de veldjes met vanggewassen lag de dichtheid tussen 1,9 en 2,8 bremraapplanten per m<sup>2</sup>. De resultaten kunnen gedeeltelijk voortkomen uit het groenbemestingseffect van het vanggewas, maar de gegevens van de laboratoriumexperimenten wijzen erop, dat zij althans ten dele kunnen worden toegeschreven aan een verlaging van de zaadvoorraad in de bodem door het vanggewas. Het ene vanggewas kan daarbij efficiënter zijn dan het andere t.g.v. verschillen in worteldichtheid, of worteloppervlakte-duur, in de productie van exsudaten, of in de hoeveelheid organische stof die wordt ondergeploegd.

#### *Rol van herbiciden in de beheersing van *Orobancha cernua**

Hoofdstuk 5 beschrijft het onderzoek naar de mogelijkheden om met herbiciden bremraap te bestrijden. In een eerste screeningsproef werd de werking van 15 stoffen getest. Uit deze middelen werden glyfosaat, imazaquin, imazethapyr en EPTC geselecteerd voor verder onderzoek. Zij werden op twee tijdstippen en in twee doseringen toegediend en vergeleken met een controle, waarin het onkruid niet werd bestreden, en een controle die met de hand werd schoongehouden. Vooral glyfosaat en imazaquin bleken effectief. Bij hogere doseringen van alle herbiciden was de schade aan het gewas echter ook aanzienlijk. Geen van de chemische behandelingen kon de opbrengst van de met de hand gewiede veldjes evenaren. De opbrengsten waren bij de lagere doseringen echter duidelijk hoger dan bij de onbehandelde controle. De bremraapopbrengst was voor de niet gewiede controlebehandeling 18,6 g/m<sup>2</sup>; na behandeling met herbicide lag deze tussen 2,8 en 10,1 g/m<sup>2</sup>.

In een andere veldproef werd aangetoond dat het systemische bladherbicide MH (Maleine hydrazide) bremraap goed kon onderdrukken bij een concentratie van 0,25 - 0,75 kg a.s./ha (Hoofdstuk 6). Bij de laagste dosering werd de hoogste tabaksopbrengst gevonden. Deze opbrengst was ongeveer gelijk aan de opbrengst die verkregen werd in de behandeling met handwieden. Bij de hogere doseringen trad gewasschade op. Het moment van toepassing bleek niet erg kritisch.

#### *Effecten van plantaardige oliën op jonge bremraapscheuten*

In Hoofdstuk 7 wordt beschreven hoe verschillende plantaardige oliën werden

getest op hun vermogen bremraap te doden. Met behulp van een druppelaar werd 0,09 ml olie per scheut toegediend. Vervolgens werd waargenomen hoe lang het duurde voordat de jonge scheut afstierf, dan wel in welke mate de bestrijding effectief was. Vooral de olie uit zaden van kokosnoot en zonnebloem gaf goede resultaten. Binnen enkele dagen stierf de jonge scheut volledig af. Er werd geen schade aan de tabak waargenomen.

### *Conclusies*

Tabak wordt ernstig belemmerd in zijn groei door bremraap. Dit effect uit zich voornamelijk in een afname van de groeisnelheid, terwijl de groeiduur vrijwel gelijk blijft. De opbrengstreductie hangt o.a. af van het aantal bovengrondse scheuten die zich per plant ontwikkelen. Het probleem kan in de toekomst wellicht worden gereduceerd door toediening aan de bodem van chemische stoffen die de kieming stimuleren in afwezigheid van een waardplant, of het telen van vanggewassen dan wel een combinatie van beide. Positieve effecten van vanggewassen werden immers ook nog gevonden na toediening van stimulantia.

Een andere weg is de inzet van herbiciden. Glyfosaat en imazaquin kunnen bremraap onderdrukken, zonder al te veel nadelige effecten voor de tabak. Met de inzet van deze herbiciden kon echter nooit het opbrengstniveau gehaald worden dat met handwieden mogelijk is. Ook een eenmalige behandeling met MH kan geen oplossing bieden omdat ook een lage dosis al snel kan leiden tot gewasschade.

Plantaardige oliën bleken de parasiet te kunnen doden zonder schade aan het gewas te veroorzaken. Deze behandeling is echter zeer tijdrovend, omdat elke bovengrondse scheut moet worden geraakt.

Geen van de onderzochte methoden bleek op zich afdoende. Daarom moet een geïntegreerde benadering van het bremraapprobleem worden ontwikkeld. Zo'n benadering zou kunnen bestaan uit de teelt van een vanggewas, gevolgd door een herbicidebehandeling in het tabaksgewas; later in het groeiseizoen kunnen door handmatig wieden of met toediening van plantaardige oliën de laatste bremraapscheuten nog worden verwijderd om te voorkomen dat produktie van nieuw zaad plaatsvindt en aldus te bewerkstelligen dat de zaadvoorraad in de grond wordt uitgeput.

## CURRICULUM VITAE

Grama Nanjappa Dhanapal was born on 20th May 1959 at Shanti Grama, Karnataka State, India. He obtained a B.Sc. (Agri) degree in 1981 and an M.Sc. (Agri) in Agronomy post-graduate degree in 1983 from the University of Agricultural Sciences, Bangalore, India. He served in the National Agricultural Extension Project of the Karnataka State Department of Agriculture from 1983 to 1988 and joined the services at the University of Agricultural Sciences, Bangalore in 1988. He worked in various capacities such as Farm Superintendent, Associate Professor (Forestry) under the National Afforestation and Eco Development Board of the Govt. of India and as Junior Breeder in the All India Coordinated Project on Oil Seeds. His tasks include research, teaching and extension until today. He was also involved in the breeding work and release of a finger millet variety; MR-2. The author has 5 research papers and 22 popular articles at his credit and (co-)authored one book and 17 publications of the Institute. The research described in this thesis was conducted at the Agricultural Research Station, Nipani, India during 1992 to 1995 for the fulfilment of the Ph.D. degree under the 'Sandwich' fellowship programme of the Wageningen Agricultural University, Wageningen, The Netherlands.