# Chapter II

# Characterization of Time of Attachment of *O. aegyptiaca* to Roots of Tobacco

## II.1 ABSTRACT

One of the difficulties inherent in studying early events in *Orobanche* parasitism of its host is the uncertainty in knowing when a given parasite actually began the process of penetrating the host. Although germination stimulants can be used to synchronize *Orobanche* germination to a certain extent, variations may occur in the time of radicle emergence and in the time needed for the parasite radicle to contact the host root. It would be useful to be able to predict tubercle age based on size or morphological characteristics, but detailed accounts of *Orobanche* development are not available. In this study, *O. aegyptiaca* tubercle growth was analyzed by stimulating parasite germination at day zero and then measuring average increases in tubercle sizes on infected tobacco plants over a fourteen day period. Results indicated that based on average increases in tubercle sizes, *O. aegyptiaca* parasitization can be divided into three phases. Phase I involves initial contact and attachment to the host root and is characterized by tubercles 0.15-0.22 mm in size. Phase II is the period in which *O. aegyptiaca* begins to withdraw resources from the host plant and is characterized by an increase in tubercle width, ranging from 0.23-0.60 mm while Phase III involves substantial increases in tubercle sizes (0.61 mm or larger) and includes the transition to a spider-like appearance. Based on these results, time of tubercle attachment does correlate with parasite development and tubercle size holds the potential to predict the time period in which an *O. aegyptiaca* attachment occurred.

## II.2 INTRODUCTION

*Orobanche* spp. (broomrapes) are parasitic plants that lack the ability to photosynthesize and are thus totally dependent on a host plant for all water, carbon, and mineral nutrients. This parasite is found primarily in semiarid tropical regions of the world and attacks many vegetable crops, grain legumes, and sunflower (Parker and Riches, 1993; Musselman, 1980). *Orobanche* reduces crop value and yield by diverting resources from the host plant to the parasite.

The *Orobanche* seed is small (0.3 mm) and contains only enough storage material to sustain the parasite during dormancy and germination (Joel and Losner-Goshen, 1994). For germination to occur, the *Orobanche* seedling must receive a chemical signal exuded from the roots of a nearby host plant. This signal initiates the emergence of a radicle that

elongates via cell expansion in the direction of the host root (Mussleman, 1980). Since the radicle can only extend a few millimeters, before reserves are exhausted, the requirement of a germination stimulus ensures that a suitable host root will be available for attachment within a few millimeters of the germinated seed.

After germination, the survival of *Orobanche* is entirely dependent on the ability of the seedling to successfully attach to a host plant. This takes place when the parasite contacts a host root, at which time the parasite radicle adheres to the root by a secreted, mucilaginous substance and produces lytic enzymes to aid in penetration of the host root tissue (Ben-Hod *et al*., 1993; Joel and Losner-Goshen, 1994). *Orobanche* appears to use a combination of enzymes including pectin methyl esterase and polygalacturonase to loosen the adhesions between host root cells, allowing the haustorium to grow between the cells (Graham *et al*., 1993; Losner-Goshen *et al*., 1998).

The haustorium is the key organ in parasitization, connecting *Orobanche* vascular tissue with host xylem and phloem tissues, and thereby allowing the parasite to act as a sink and withdraw carbon, water, and mineral nutrients from the host (Dörr, 1996). As water and nutrients are obtained from the host plant, the parasite tissue outside the host root swells into a bulbous mass called as a tubercle. The tubercle continues to accumulate nutrients until it produces a floral shoot. This shoot is the only above ground structure of the parasite and can produce up to 200,000 seeds (Parker and Riches, 1993).

As *O. aegyptiaca* penetrates and forms connections to the host vascular tissue, the host reacts to this invasion with attempts at self-defense (Westwood *et al*., 1998). In order to fully understand these host responses, it would be useful to be able to predict tubercle age based on size or morphological characteristics. Although germination stimulants can be used to trigger a synchronized germination, variations may occur in the time of radicle emergence and subsequent contact with the host root. The objective of this experiment was to analyze *O. aegyptiaca* tubercle growth on infected tobacco in order to determine if changes in size or morphology can be used in predicting the time at which a parasite attachment occurred.

## II.3 MATERIALS AND METHODS

### II.3.1 Plant Growth Conditions

Tobacco (*Nicotiana tabacum* L. var. Coker) were grown from seed in soil for approximately 12 days, at which time 9 tobacco plants were transplanted into each polyethylene (PE) bag [as described in Westwood et al. (1996)] containing glass fiber filter paper and watered with 0.5X Hoagland solution (Hoagland and Arnon, 1950). Plants were grown under 100 µmol/$m^2$/sec light, 12 hr. days at 25 ± 3°C. *O. aegyptiaca* seeds were surface sterilized using 70% EtOH and 1% sodium hypochlorite. Seven days after transplanting, sterilized *O. aegyptiaca* seeds were applied to tobacco roots using a small paint-brush. Seeds were brushed around and against roots to form a nearly continuous band. Seed were placed only on roots sections located 1 cm down from shoots and 1 cm up from the bottom of the bag. Numerous seeds were applied in order to obtain a maximum number of attachments. The *O. aegyptiaca* seeds which require a period of pre-conditioning prior to germination, were then allowed to precondition on the tobacco roots for seven days. At the end of this time, GR-24 (1mg/L), a known germination stimulant for *Orobanche* (Mangnus *et al*., 1992), was

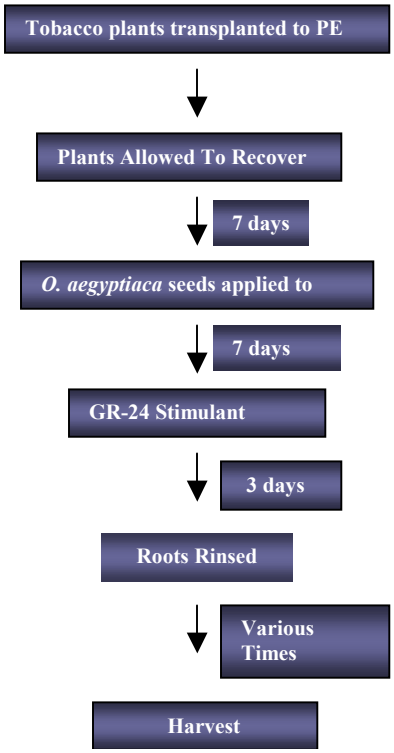


Figure II.1: Diagram indicating sequence of events in plant growth and treatments.

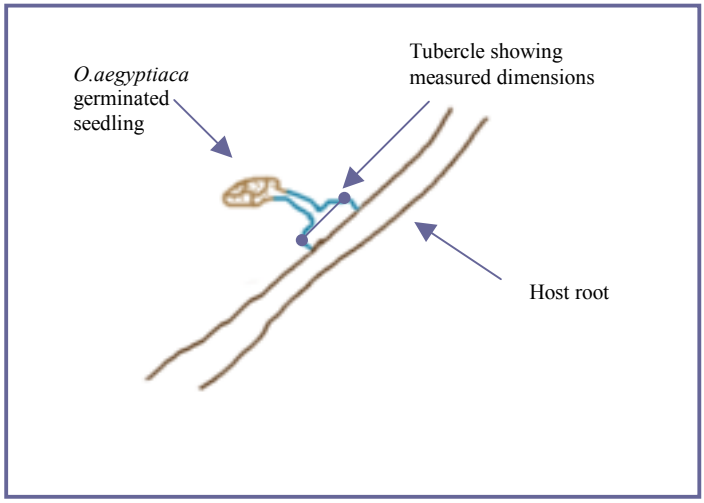


Figure II.2: Illustration depicting part of a root section containing *O. aegyptiaca* attached to a host root. The width of the turbercle (as indicated by the bulleted line) was measured under a stereo-zoom microscope.

injected into the back of each PE bag using a 10mL syringe, and three days were allowed for parasite germination and radical contact with the host root. Three days after application of GR-24, unattached *O. aegyptiaca* seeds were removed from host roots by gently rinsing roots with a stream of distilled water. Plants were placed back under light as described earlier and Hoagland solution was added as necessary to ensure complete saturation of the filter paper. Plants were divided into six groups each containing three replicate bags which were harvested at 5, 6, 8, 11, 14, or 18 d after addition of GR-24 for experiment 1 and 3, 6, 8, 10, 13, or 17 d for experiment 2. At each time point, eight to ten tubercles from each of the three bags were randomly selected, measured cross-sectionally (Fig. II.2), and photographed under a stereo-zoom microscope. Daily averages and standard deviations were calculated.

## II.4 RESULTS AND DISCUSSION

The use of polyethylene bags allows in-situ monitoring of *Orobanche* parasitization of host plants and is a common technique for studies of parasitic plants (Parker and Dixon, 1983; Goldwasser *et al*., 1997). The objective of this experiment was to use this system to try to draw a correlation between tubercle size and time of parasite establishment on the host root.

Previous experiments have indicated that *O. aegyptiaca* seeds require at least three days for germination and radicle contact of the host root in the PE bag system (Westwood *et al*., unpublished data). The width of the radical at the time of contact with the host root is approximately 0.15 mm. This dimension remains the same for about four days before it starts to swell into a tubercle (Fig. II.3). The tubercle then gradually enlarges for approximately 6 days. Around 13 days after application of GR-24, tubercle growth increases dramatically and by 18 days has increased 2-4 fold in size and the shape of the tubercle has become “spider-like”. Based on this data, *O. aegyptiaca* development can be divided into three phases: Phase I consists of tubercles ranging in size from 0.15-0.22 mm and represents tubercle growth 3-7 days after addition of GR-24. In Phase II tubercles are 0.23- 0.60 mm in diameter and 7-14 days after addition of GR-24. Phase III represents tubercles 0.61 mm or larger and reflects tubercles older than 14 days after GR-24 application.

Division of early *O. aegyptiaca* development into three phases likely reflects underlying biological phenomena. During Phase I (Fig. II.3) *O. aegyptiaca* is secreting enzymes to penetrate the host tissue and establish a connection with the host plant (Losner-Goshen *et al*., 1998). This haustorial penetration induces the expression of plant defense genes in the region of parasitization (Westwood *et al*., 1998; Joel and Portnoy,

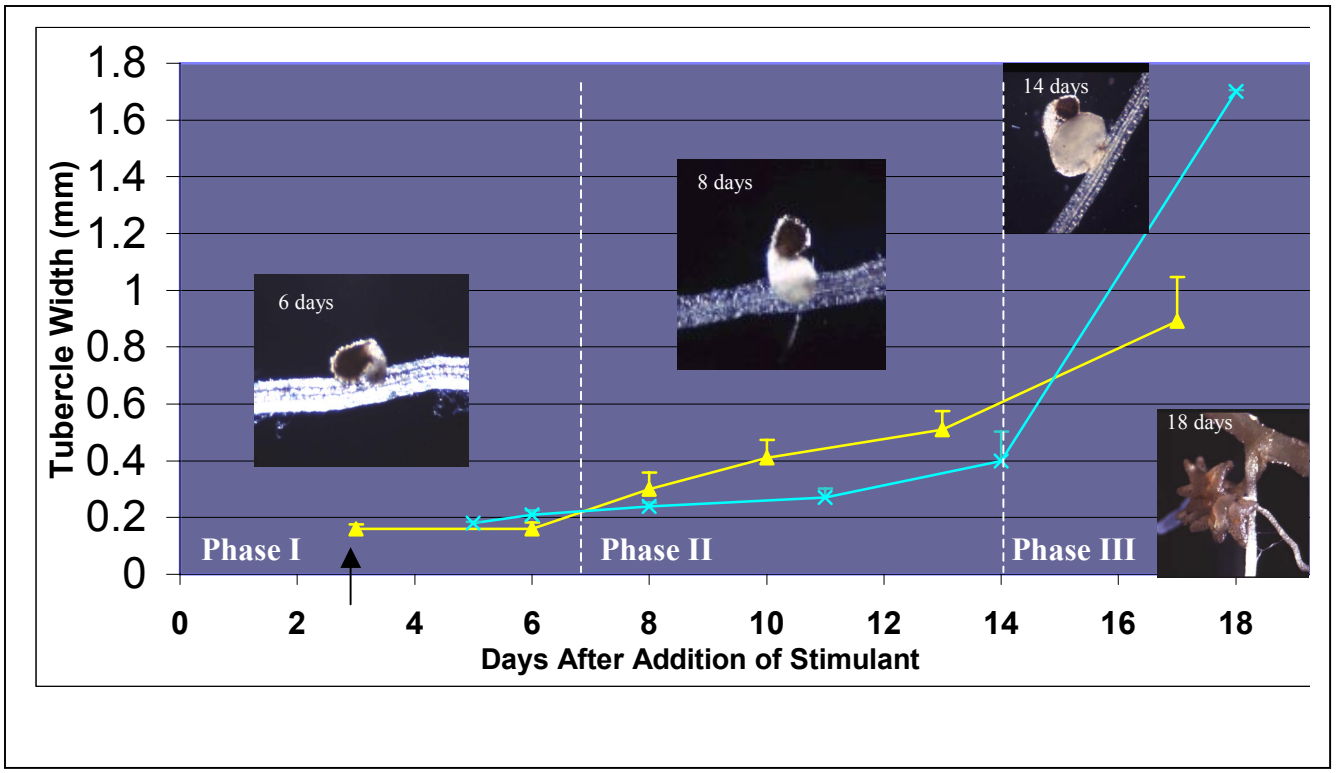


Figure II.3: Growth of *O. aegypticaca* tubercles on tobacco roots. Data from experiment 1 (▲) and experiment 2 (x) indicate mean widths of parasite tubercles on roots. Arrow at day 3 indicates time of attachment and time at which roots were rinsed to remove unattached tubercles. Graph is divided into Phase I, representing 0-7 days, Phase II, 7-14 days, and Phase III, 14-18 days after triggering germination by addition of GR24. Standard deviations of means are indicated by vertical lines. Examples representative of parasites at each stage are shown.

1998; Griffitts *et al*., unpublished data). No substantial tubercle growth occurs during this phase, however, as this appears to be the time period in which the parasite forms a vascular attachment to the host plant. This stage is critical for the parasite and determines its future development and survival.

This is also an important period for the host because the parasite appears to be involved in a lengthy process of establishing itself, but has little resources of its own. Plant defenses during this time frame should be effective in stopping parasitism.

During Phase II, increases in tubercle size indicate that the parasite has successfully connected to the host plant and is withdrawing water and mineral nutrients. At this stage, *O. aegyptiaca* is functioning as a true holoparasite. The slow increase in size suggests that the tubercle is acting as a weak sink and may still be augmenting the haustorial connections. Once the parasite has been attached to the host plant for approximately 14 days, the tubercle growth rate increases (Fig. II.3). In Phase III, the parasite is likely extracting larger quantities of water and nutrients from the host. Furthermore, by day 18, *O. aegyptiaca* has changed shape and the tubercle now possesses secondary roots (Fig. II.3). These roots will continue to grow and may be capable of forming secondary attachments to hosts. A floral meristem will then develop, giving rise to a floral spike.

These results have provided useful information concerning the timing of events in *O. aegyptiaca* parasitization. From these two experiments, it is evident that in tobacco, tubercle growth is generally consistent and reproducible. However, variation in growth, combined with the extremely slow early growth rate, raises questions about how accurately tubercle size can be used to predict the time at which a parasite attachment occurred. While pinpointing the time of attachment to a specific day may be difficult, classifying tubercles into one of the three phases is possible and carries biological meaning. In the experiments described here, care was taken to prevent *O. aegyptiaca* attachments from occurring any time but day 3. For experiments on gene expression in host roots, such precautions would be laborious and lead to very few attachments. Rather, plants for expression studies were allowed to be parasitized indiscriminately following GR-24 addition. Thus many attachments would form at 4-7 days after stimulant addition, as well as 3 days. Plants harvested at 10 days after GR-24 addition would thus contain a mixture of late Phase I and early Phase II tubercles. Although not completely homogenous, these share the characteristics of being in the process of establishing themselves in the host tissue.

Although tubercle growth was only analyzed on roots of tobacco a similar approach could be used to determine the age of parasite attachments on additional host plants. The length of the phases and rate of tubercle growth may be independent of the host or may be proportional to the size of the host plant, by which this data could be scaled up or down for application to larger or smaller host plants such as Sunflower or Arabidopsis. Additional experiments involving the analysis of tubercle growth on such host plants is needed.

## II.5 ACKNOWLEDGEMENTS

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## II.6 REFERENCES

Ben-Hod G., Losner D., Joel D.M., and Mayer M. 1993. Pectin methylesterase in calli and germinating seeds of *Orobanche aegyptiaca*. *Phytochemistry* 32:1399-1402.

Dörr, I. 1996. New results on interspecific bridges between parasites and their hosts. In: *Advances in Parasitic Plant Research*, (M.T. Moreno, J.I. Cubero, D. Berner, D. Joel, L.J. Musselman and C. Parker, eds.), 195-201. Junta de Andalucia, Cordoba, Spain.

Goldwasser, Y., Kleifeld, D.P., and Rubin, B. 1997. Variation in vetch (*Vicia* spp.) response to *Orobanche aegyptiaca*. *Weed Science* 45:756-762.

Graham, B.H., Losner, D., Joel, D.M., and Mayer, A.M. 1993. Pectin methylesterase in calli and germinating seeds of *Orobanche aegyptiaca. Phytochemistry* 32:6:1399-1402.

Hoagland D.R. and Arnon D.I. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 347:1-32.

Joel, D.M. and Losner-Goshen, D. 1994 The attachment organ of the parasitic angiosperms *Orobanche cumana* and *O. aegyptiaca* and its development. *Canadian Journal of Botany* 72:564-74.

Joel, D.M. and Portnoy, V.H. 1998. The angiospermous root parasite *Orobanche* L. (*Orobanchaceae*) induces expression of a pathogenesis related (PR) gene in susceptible tobacco roots. *Annals of Botany* 81:779-781.

Losner-Goshen, D., Portnoy, V.H., Mayer, A.M., and Joel, D.M. 1998. Pectolytic activity by the haustorium of the parasitic plant *Orobanche* L. (*Orobanchaceae*) in host roots. *Annals of Botany* 81:319-326.

Mangnus, E.M., Stommen, P.L.A., and Zwanenburg, B. 1992. A standardized bioassay for evaluation of potential germination stimulants for seeds of parasitic weeds. *Journal of Plant Growth Regulation* 11:91-98.

Musselman, L.J. 1980. The biology of *Striga, Orobanche*, and other root-parasitic weeds. *Annual Review of Phytopathology* 18:463-489.

Parker, C. and Dixon, N. 1983. The use of polyethylene bags in the culture and study of *Striga* spp. and other organisms on crop roots. *Annals of Applied Biologists* 103:485-488.

Parker, C. and Riches, C.R.1993. Parasitic Weeds of the World: Biology and Control. Cab International, UK.

Westwood, J.H., Yu, X., Foy, C.L., and Cramer, C.L. 1996. Parasitization by *Orobanche* induces expression of a defense-related gene in tobacco. Pages 543-550 in: Advances in Parasitic Plant Research. M.T. Moreno, J.I. Cubero, D. Berner, D. Joel, and L.J. Musselman, eds. Junta de Andalucia, Cordoba, Spain.

Westwood J.H., Yu X., Foy C.L., and Cramer C.L. 1998. Expression of a defense-related 3-hydroxy-3-methylglutaryl CoA reductase gene in response to parasitization by *Orobanche* spp. *Molecular Plant Microbe Interactions* 11:530-536.