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Developmental Mechanisms of Parasitism in Broomrape (*Orobanche Spp.*) and Their Potential Impact on Control

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Developmental Mechanisms of Parasitism in Broomrape (*Orobanche* spp.) and Their
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**DEVELOPMENTAL MECHANISMS OF PARASITISM IN BROOMRAPE
(*OROBANCHE* spp.) AND THEIR POTENTIAL IMPACT ON CONTROL**

Final Report

Submitted to

BARD - United States-Israel Agricultural Research and Development Fund

by

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Abstract

Orobanche is one of the most important agricultural pests in Israel. Existing methods for its control are generally not practical or economic. A mechanistic understanding of this root-parasite is required to search for methods for its control.

A germination stimulant was purified from flax root exudate. Its specific activity toward *O. aegyptiaca* was more than ten-fold higher than that of strigol, the most potent known stimulant. Stimulants from flax and sunflower were relatively specific for *O. aegyptiaca* and *O. cumana*, respectively. They differed in polarity and chromatographic properties.

Structural studies of early *Orobanche* development led to several new observations: (1) The parasite anchors itself to host surface by means of a carbohydrate secretion which is released through special wall structures on the outer side of haustorial papillae. (2) When *Orobanche* seedling develops a haustorium it shifts from lipid accumulation to the accumulation of starch. This was utilized as simple assay for initiation of haustoria. (3) Primary haustoria in *Orobanche* initiate spontaneously *in vitro* in the absence of any host or haustorial inducer, contrary to other parasites like *Striga*. (4) Bacteria are commonly found on surface and in outer walls of *Orobanche* seedlings.

An axenic model-system for the study of the infection process was developed, including: (1) aseptic conditions for germination, (2) growth of callus in solid and liquid cultures, (3) differentiation of callus protrusions resembling roots, and (4) infection of host roots with callus protrusion *in vitro*. This system was used for biochemical studies. Wall degrading enzymes were found in calli and seedlings and immunocytochemically detected in the infection zone.

d) Objectives of the original research proposal

The original objectives of the investigation were addressed as follows:

- (1) Isolate and characterize germination inducer(s) for *Orobanch* from host root exudates. Determine relevance of root exudate chemistry and germination stimulation to host specificity.
- (2) Conduct detailed anatomical and ultrastructural studies of early *Orobanch* development. Identify morphological and/or histochemical markers for assay of haustoria induction.
- (3) Determine the biochemical basis of haustoria induction in *Orobanch*. Purify and identify inducers. Explore role of haustoria induction in host specificity.
- (4) Compare resistant and susceptible hosts to understand mechanisms of parasitism and resistance. Analyze biochemical and ultrastructural events in haustorial development and penetration in resistant/tolerant species.

Deviations from the original objectives:

Although support by BARD was approximately half the requested budget, we were able to achieve considerable progress in most objectives. As a subsidiary objective we also planned to investigate possible enzymes required for host penetration by *Orobanch*, and progress was made in this area as well (see pp. 73,76).

- (1) Although we have purified a preparation of flax stimulant 58,000-fold, there was still not enough material to establish purity. The task of obtaining enough of this material for preliminary structural analysis remains daunting. Only then would it be possible to determine relevance of root exudate chemistry to host specificity. Nevertheless this degree of enrichment was sufficient to show that the flax stimulant causes half-maximal germination of *O. aegyptiaca* at a concentration of 30 pM or less, a specific activity that is more than ten-fold higher than that of strigol or GR-24, the most potent known germination stimulants for any *Orobanch* species. The exceptionally high activity of the flax stimulant suggests that this stimulant may be nearly pure. The next step to be done is to scale up the preparation, as detailed in our new research proposal.

(3) The biochemical basis of haustoria induction in *Orobanche*: We found out that contrary to parasites of the Scrophulariaceae like *Striga*, in *Orobanche* the primary haustorium initiates spontaneously *in vitro*, i.e. it initiates in the absence of any host or haustorial inducer (see p. 39). Further to the hypothesis that laccases might be responsible for the release, in neighboring roots, of substituted quinones capable of stimulating the initiation of parasitic haustoria as shown for both *Striga* and *Agalinis*, we have shown that *Orobanche* calli contain only catechol oxidase activity and no laccase (see p. 84). For these reasons isolation of a haustorial inducer was not relevant, and was not further studied.

(4) A major problem in the study of haustorial penetration through host tissues is the presence of bacterial spores in the infection site (see p. 41). Therefore, instead of pursuing the objective as planned we developed, during the first two years, an *in vitro* axenic system for growth of calli and infection of intact host plants with callus protrusions (see pp. 57, 61, 65). Once the model-system was available to us, we started both the structural study (see p. 48) and the enzymological study of penetration (see pp. 73, 76).

Cooperation

The American effort was focused on germination stimulation and host root exudates, and the Israeli effort primarily directed toward understanding post-germination events: developmental aspects in Newe-Ya'ar, and physiological and biochemical aspects in Jerusalem.

The two Israeli teams worked in close collaboration, using the same plant material and sharing facilities. D. Losner-Goshen, the Ph.D. student under joint supervision of DMM and AMM bridged between the two labs on a weekly basis. AMM and DMJ have met at least once every month. G. Ben-Hod (of the Jerusalem Team) and DLG also collaborated in the enzymatic work. The *in vitro* model system is the result of collaboration between AMM and DMJ.

Communication was also open between the Israeli team and that in the US, using electronic mail, fax and telephone throughout the research period, so that all current results in both countries have been dealt with and discussed on the spot.

Dr. Steffens has visited Israel for two weeks (Winter 1990). He stayed in Newe Ya'ar not only to discuss current results, but also to learn methods of *Orobanch* research employed in the *Orobanch* lab. In addition Dr. Steffens visited the department of Botany in Jerusalem for further discussions with Prof. Mayer and his team. All investigators then had the opportunity of jointly going to a field excursion and learning of the natural and field habitats of different parasites of the *Orobanchaceae* and *Scrophulariaceae*, as well as of other parasitic plants in different parts of the country including the desert. Prof. Mayer met Dr. Steffens in the U.S. in summer 1989. Dr. Matthews (of the Cornell team) and DMJ have met during the 5th International Symposium of Parasitic Weeds in Nairobi (summer 1991) discussing current results and presenting joint papers. DMJ spent his sabbatic leave at JCS's lab at Cornell (August 1991 - October 1992), and worked jointly on parts of the project.

Seeds of *Orobanch aegyptiaca* and *O. cumana* were sent to Cornell University from Newe Ya'ar. Seeds of the same batches were also given to the team at the Hebrew University of Jerusalem. All three labs also worked on the same host seed-stocks.

Plant material which was grown in culture in Jerusalem was frequently examined in Newe Ya'ar. Plant extracts obtained in Jerusalem were assayed for activity on *Orobanch* seed germination in Newe Ya'ar. Young *Orobanch* seedlings and plants grown in Newe-Ya'ar served in experiments in Jerusalem. Transmission EM of material prepared in Newe-Ya'ar was carried out in Jerusalem.

Evaluation of the research achievements

Broomrape (*Orobanch* spp.) is a parasitic weed of worldwide distribution, attacking the roots of various crops and causing extensive damage. Infestation with this parasite often reduces the yield and in severe cases leads to a complete crop failure. Although some methods which are currently used for the control of parasitic weeds belonging to the genus *Orobanch* (the broomrape) have encountered various degrees of success, they are generally ineffective or prohibitively costly. With certain crops and soil types these methods are often not applicable.

This project was aimed to contribute a mechanistic understanding of parasitism by *Orobanch* as a basis to search for new methods for its control and eradication. It was also aimed to throw light on recognition processes in parasitic weeds.

Work on the development of *Orobanch* seedlings and haustoria, revealed anatomical, morphological and histochemical criteria for the identification of the different developmental phases. We obtained, for the first time, an insight of surface characteristics of the attachment surface of *Orobanch* and achieved an understanding of the mode of attachment of the haustorium to the surface of the host root. The event of attachment signals a kind of metamorphosis of the young *Orobanch* from independent to parasitic phase. It was found that this change includes a shift from the accumulation of lipids, which is typical of the embryo to the accumulation of starch which proceeds throughout the parasitic phase. This novel finding can be exploited in any bioassay aimed to detect the first signs of haustorial initiation. The understanding of the various stages of seedling growth, haustorium attachment to host root, and penetration of intrusive haustorial cells through host tissues is required for the precise enzymological, immunocytochemical and histochemical studies of these events.

Some work has also been done on new inhibitors of *Orobanch* germination and on culture conditions which cause the breakdown of infection of infected plants.

The most significant methodological achievement in our post-germination research was the development of an in-vitro axenic model-system for growth of *Orobanch* from callus and for infection of intact host roots under aseptic conditions. *Orobanch* plants which develop from these infections proved to be normal both in structure (including the structure of the haustorium) and in development. With this method we were able to prove that infection by *Orobanch* is possible without the presence of microorganisms. This model system facilitates the study of enzymatic activities by the parasite and the host in the infection zone without the interference of bacterial enzymes. Using this system we were able to demonstrate enzymatic activities of polygalacturonase and pectin methyl esterase, two important enzymes which might

be used by the parasite for penetration in host tissues.

We have isolated a natural germination stimulant from flax root exudates. The flax stimulant caused half maximal germination of *O. aegyptiaca* at a concentration of 30pM or less. This specific activity toward *O. aegyptiaca* is more than ten-fold higher than that of strigol or GR-24, which are the most potent germination stimulants known hitherto for any *Orobanch*e species. The exceptionally high activity of the flax stimulant is significant because it is the first highly potent stimulant to be isolated from a natural host (flax is heavily parasitized by *Orobanch*e *aegyptiaca* in certain parts of Israel). If purified and identified, this stimulant, or its derivatives, might be used for the development of effective germination stimulants for use in the field as false germinators, and/or to the development of specific inhibitors of broomrape germination. The use of these substances might contribute to further research aimed to examine the mechanism by which *Orobanch*e seeds respond to germination stimulants.

List of Publications

Ben-Hod, G., Losner, D., Joel, D. M. and Mayer, A. M. 1991. In Vitro culture of *Orobanch*e *aegyptiaca*. Ann. Bot., 68:413-416

Ben-Hod, G., Losner, D., Joel D. M. and Mayer, A. M. 1991. Infection of tomato roots using calli of broomrape. Physiologia Plantarum, 83:353-356.

Matthews, D.E., Joel, D.M., and J. C. Steffens (1991). Species specific responses of *Orobanch*e to host-derived and synthetic germination stimulants. Proceedings Fifth International Symposium of Parasitic Weeds, Nairobi, Kenya. pp 428-434.

Ben-Hod, G., Losner, D., Joel, D. M. and Mayer, A. M. 1993. Pectin methylesterase in calli and germinating seeds of *Orobanch*e *aegyptiaca*. Phytochemistry, 32:. (In Press)

Losner-Goshen, D., Joel, D.M., Mayer, A.M. and Steffens, J.C. 1991. Surface characteristics and internal structure of *Orobanch*e seedlings. Proceedings Fifth International Symposium of Parasitic Weeds, Nairobi, Kenya. pp 356-360