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DEPARTMENT OF THE ARMY

Fort Detrick
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CHOKE-WEEDS: I - EXPERIMENTAL CULTURES

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Summary: Description of simple, effective method permitting germination and growth of choke-weeds on the roots of their hosts. Positive results in nine of twelve strains studied.

The first stages of the parasite can easily be observed in vitro.

1. INFORMATION ABOUT THESE PARASITES

Choke-weeds, parasitic plants of higher vegetable plants, are for the most part unknown to the general public. Their color and appearance keep them from being used in flower bouquets, but it sometimes happens that their abundance intrigues the layman.

Here and there, however, they are formidable enemies of crops: around the Mediterranean, Orobranche crenata Forsk. exhaunts bean cultivation which in under-nourished regions is a protein food in great demand; in the USSR, O. cumana Wall. decreases the sunflower harvest; Striga sp., which is more generally classed among the Scrophulariaceae, attacks sorghum, corn and sugar cane in warm climates; Phelipaea ramosa Mey. causes hemp and tobacco to wither in Rumania and in the Loire valley in France; O. minor Sutton inflicts damage to clover fields in the same region. Nearer us, O. medicagnis Duby

*This paper was read at the meeting of 22 April 1965, submitted by Mr. Werner.*
reduces the yield of lucern fields around Pont-à-Mousson and Pont-Saint-Vincent.

The biology of these plants has been of interest to many researchers, both botanists and agronomists; in 1827 Vaucher wrote a "Monograph on Choke-Weeds" and tried to observe their germination; Koch carried on this work (1875, 1857); Partisinsky used rootlet excretions of the host in making O. cumana germinate; Schorolin (1934-1938) studied the germination of O. crenata; Brown and his colleagues (1944-1952) tried by chromatography to separate and determine those products responsible for the germination of Striga hermonthica, S. lutea and O. minor; Izard and his colleagues studied Phelipaea ramosa and tried to find agents capable of making it germinate without a host in order to destroy it in crops; Racovita works on the same species in Rumania and sought plants that make it germinate without serving as host; Kadry and Tewfic study the germination and development of O. crenata; Privat (1960) collected many studies of O. hederae on its host Hedera helix and on their relations.

2. EXPERIMENTAL REQUIREMENTS

Studying the role of rootlet excretions of plants in nature, it seemed very interesting to us to know the action of different phanerogams that can be parasitized on 12 strains of choke-weed.

To successfully carry out our work it was necessary both to be able to follow the germination and the first stages of attachment of the parasites and also to make a collection of them in order to observe their biological cycle.

For the seeds of the parasite to germinate, they must locate themselves in the rhizosphere of the host or receive the products excreted by the roots; very rare germinations were, however, observed in the absence of a host, in moist sand -- unabrolin (1936) -- or on distilled water -- Izard (1957), Krenner (1958) -- in O. crenata, P. ramosa, O. cumana; several mineral or organic products have allowed the germination of P. ramosa -- Izard and Hitler (1953, 1958) -- and of O. hederae -- Privat (1959). For growth to take place, the procambium (germinating tube starting from the seed and carrying the embryo) must come in contact with a root: the parasite penetrates this and a mixed organ is formed, the tubercle. A true graft occurs at this same level with a reorganization of vascularization to the advantage of the parasite.

The small size of the seeds (40 to 100 μ by 150 to 600 μ) and sometimes the small volume of available amounts means that in the earth contact between the roots of the host and the seeds of the parasite is experimentally chancy.
On the other hand, natural stations benefit by the viability of the seeds (ten years and more) and of their accumulation over the years.

3. METHODS USED

Whether one is studying the first stages of the parasite or obtaining adult plants, the fixation on the host roots is done in vitro. The eventual replanting in the earth of the nourishing plants and their choke-weeds can thus be carried out later with full knowledge of the facts.

The following technique yields good results:

The host seeds are set to germinate on moistened perlite with the help of a nutritive mineral solution of Hoagland and Arnon. When the roots reach 5 to 7 centimeters length, the sprouts are replanted individually in test tubes 25 centimeters long, 25 mm in diameter, a third filled with the above nutritive solution. A rubber stopper with an 8 mm opening holds each sprout at the level of the neck. The roots sit on the surface of a thin glass plate covered by a strip of filter paper immersed in the solution. The nutrition of the sprout and the aeration of the root system are thereby ensured. The test tubes are then placed in opaque supports.

After a good redevelopment of the host, the seeds of the parasite are distributed at the periphery of the roots, touching them. A preliminary washing of the seeds allows elimination of brown, tegumentary substances harmful to the young roots. This last procedure is carried out in a Swiny filter unit, the seeds being placed between two Durieux No. 268 filters and rinsed by a stream of distilled water.

Ten to twenty days later the seeds germinate and a month after being set in place young tubercles due to the parasite are visible.

When the tubercles reach 8 to 10 mm size, the procedures differ depending on the goals in view:

a) if one wishes to obtain young tubercles whose pseudoroot spread does not exceed 20 to 25 mm, the thin glass plate and strip of paper are removed, the nutritive solution is replaced by a fresh medium, the level of which is brought to slightly under the insertion point of the first tubercle.

b) if a more advanced stage or adult individuals are sought, the parasited plants are replanted in pots containing fine-grain soil.
c) if the final goal is making a collection, the transfer-
ral to pots is followed by a final planting two to three months
later.

1 -- Host; 2 -- Stopper (empty tube); 3 -- Parasite tuber; 4 --
Parasite roots and seeds; 5 -- Thin glass plate; 6 -- Filter
paper; 7 -- Nutritional liquid; 8 -- Culture arrangement; 9 --
Durieux filters No. 268; 10 -- Rubber gasket; 11 -- Placement
of seeds; 12 -- Screen; 13 -- Swinny apparatus (assembled for
washing of the seeds).

4. RESULTS

Positive results can be obtained with:

<table>
<thead>
<tr>
<th>Orobanche epithymum D.C.</th>
<th>on Thymus serpyllum L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. crvngt, Duby</td>
<td>on Eringium compositum L.</td>
</tr>
<tr>
<td>O. glau Duby</td>
<td>on Galium mollugo L.</td>
</tr>
<tr>
<td>O. hederae Duby</td>
<td>on Hedera helix L.</td>
</tr>
<tr>
<td>O. major L.</td>
<td>on Centaurea scabosa L.</td>
</tr>
<tr>
<td>O. minor Sutton</td>
<td>on Trifolium repens L.</td>
</tr>
<tr>
<td>O. picrida Schulz</td>
<td>on Piers hieracoides L.</td>
</tr>
<tr>
<td>Pheum caerulea Mey.</td>
<td>on Achilles millofolium L.</td>
</tr>
<tr>
<td>P. ramosa Mey.</td>
<td>on Trigonella aemum-graeeum L.</td>
</tr>
</tbody>
</table>

On the other hand, the parasite did not germinate in the
presence of its usual host in the cases of:
In each of these three cases the seeds of the parasite may not germinate until an advanced stage of vegetation of the host plant, for example flowering for O. crenata Forsk, which parasites beans as Kadry and Tewfic pointed out.

5. CONCLUSIONS

This method can facilitate the biological study of root-parasitic phanerogams: by supplying a pure, intact body the parasitic stages of which are difficult to harvest on the land; by allowing regular observation of the growth of the parasite; by facilitating the study of certain mechanisms of parasitism (germination of the seeds, resistance of the host to invasion, modification or suppression of certain functions by degradation and adaptation to parasitic life). The fight against a parasite is more effective as its biology becomes better known.

One must note that the special characteristics of these plants makes them sought after for reasons of curiosity by many botanical gardens.

The many calls for seed reaching the Botanical Garden of Nancy since their listing in the catalogue of exchanges reflects the interest in them.

Using this method to gather a collection of such parasites avoids many failures, for 300 to 300 seeds are sufficient, whereas to have any chance of success one must mix four to five ml. of seed — in other words several million — to fine-grain soil in each pot which will receive the host seeds.

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