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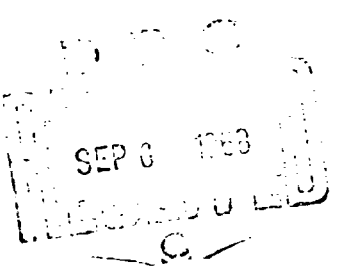

THE GERMINATION OF CHOKEWEED SEEDS IN TOBACCO (IN VITRO)

Academie d'Agriculture de France,
Comptes Rendus (Academy of Agriculture of France, Reports), Vol 39, 1953, pages 567-569.

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The branchy chokeweed (Phelipoea ramosa C.A. Mey or Orobanche ramosa L.) is a totally parasitic seed plant which can cause considerable damage to tobacco plantings. Exhausted by the parasite, the plants become etiolated, the leaves turn yellow, and production can be seriously compromised in weight as in quality.

The seed, very small (less than 0.3 mm in length and 0.2 mm in width) and produced in abundance, is easily disseminated by the wind and can preserve its germinating power for several years. The embryo, which is undifferentiated, develops if conditions of temperature and humidity are favorable and if it is in contact with a root of the host plant or in its immediate vicinity. For certain researchers this last condition is indispensable. Thus, Mastromarino [see Note] was only able to obtain the germination of the seeds of P. ramosa by sowing them next to tobacco plants transplanted in an open field.

[Note]: A. Mastromarino, Observations and Research on Orobanche of Tobacco. B. tecn. Scafati. XV, No 3, 1937.

Our own research, conducted over several years at the Experimental Tobacco Institute of Bergerac, leads to new conclusions both from the biological as well as the practical points of view.

I. Preliminary Research

Attempts at germination were carried out by placing the chokeweed seeds in Petri boxes containing soil to which saccharose may or may not have been added. No results were obtained after several weeks. Other experiments were carried out by placing the seeds on sand saturated either with solutions containing various substances capable of exercising an action: sugars, vitamin B 1; or extracts of tobacco roots and of soil originating from tobacco plantings. All these experiments were negative.

Moreover, experiments conducted under aseptic conditions in the areas of Knudson and Tuckey yielded no results.

We then tried to germinate the seeds of the chokeweed in the presence of young tobacco roots. For this purpose, a swab of cotton three centimeters thick and impregnated with distilled water was placed in a test tube, taking care to place a certain number of chokeweed seeds on the lower part of the swab and a few tobacco seeds on the upper part. A number of tubes prepared in this way were put in an oven regulated at 22°C. The tobacco seeds germinated without their roots reaching the chokeweed seeds. After twenty days, a chokeweed seed clearly germinated was observed in a tube. From this first result it was possible to conclude that contact with a tobacco root was not necessary for the germination of the seeds of the parasite. Moreover, one might think that the substance responsible for the germination came either from the young tobacco roots or from the cotton itself. As an attempt at germination on a hydrosoluble extract of combed cotton produced no results, one could assume the existence of specific substance diffused by the young tobacco roots.

II. Action of Nicotinamide (Vitamin PP), of Pyridoxine (Vitamin B 6), and of Nicotine on Chokeweed Seeds

Rather than attempt a separation by adsorption of the active component, we tried to provoke the germination of the chokeweed seeds by using chemically defined substances capable of being assimilated by the seeds of the host plant. It is in this way that we tried nicotine, nicotinamide, and pyridoxine. In all our experiments, the chokeweed seeds were placed on filter paper, fed by active solutions, and maintained at a temperature of 24-25°C.

The nicotine base used in aqueous solutions in the proportion of 1,10 and 100 milligrams to the liter never caused the germination of seeds of the parasite.

Vitamin PP was used in a first experiment in the following concentrations: 1,10 and 100 mg per liter. We obtained a great number of germinations as the following table indicates:

Nicotinamide	1 mg/liter	10 mg/liter	100 mg/liter
Percentage of seeds germinated after 24 days	3.2	18	0

In a second experiment on another lot of seeds, we obtained very high percentages of germination, as follows (the concentration of nicotinamide was 10, 50, and 100 mg of the liter):

Nicotinamide	10 mg/liter	50 mg/liter	100 mg/liter
Percentage of seeds germinated after 24 days	45.5	27.5	6.6

After thirty-four days of culture, 60 percent of the seeds fed by the solution of 10 mg/liter had germinated.

Since vitamin PP had provoked the germination of the tobacco chokeweed seeds, we tried vitamin B 6, which also possesses a pyridine nucleus.

Vitamin B 6 has a very pronounced action, as the following table shows:

Vitamin B 6	1 mg/liter	10 mg/liter	100 mg/liter
Percentage of seeds germinated after 24 days	9	8.3	1.5

Experiments conducted by mixing vitamins PP and B 6 confirmed that vitamin PP was more active than vitamin B 6.

III. Interpretation of Results

The achievement of germination in vitro of the seeds of Phelipoea ramosa contributes appreciably to the biological knowledge of the holoparasitic angiosperms. We know that most of them develop under the action of undetermined substances which are secreted by the plants affected by parasites. It is on account of this that many researchers have indicated that they have obtained the germination of seeds of certain chokeweed or figwart parasites by using radicular extracts of the host plants. M. Chabrolin [see Note], especially, obtained the germination of sowings of orobanche speciosa by treating them with radicular extracts of various leguminous plants. Therefore, the germination of seeds of Phelipoea ramosa under the influence of radicular secretions of tobacco should not be surprising.

[Note]: Ch. Chabrolin, Contribution to the Study of the Germination of Seeds of Chokeweed of the Bean, Ann. Serv. Bot. Tunisie, 1937-1938, 14-15.

Of greater interest is the evidence of the action of the vitamins with a pyridine base on the germination of the seeds of the parasite.

Indeed, this is the first time, to our knowledge, that the germination of a chokeweed has been obtained by the aid of chemically defined substances having a vitamin content. Moreover, it seems that many types of cultivated plants secrete such substances, and it must be remembered that S. Banerjee and R. Banerjee [see Note] recently demon-

strated that many of the graminaceae and leguminous plants synthesize a large quantity of nicotinic acid in the course of their germination. The specificity of a host and of a given parasitic species could result from the nature and the quantity of substance secreted as well as from environmental conditions. As regards the biochemical specificity of the substances capable of removing the inhibition of the embryos of P. ramosa, it seems established that the pyridine nucleus is indispensable. Nevertheless, its presence is not sufficient to cause germination, as in the case of nicotine. The substance must also be endowed with a vitamin content. Amide of nicotinic acid is precisely one of these substances, and it is known to intervene, particularly in the oxidation of sugars and their phosphoric esters.

[Note]: S. Banerjee and R. Banerjee, Biosynthesis of Nicotinic Acid by Germing Pulses. Ind. J. Med. Res., 1950, 38, No 2.

From the biological point of view, the results obtained call attention to the important role which vitamins can play in the vegetable kingdom and, more particularly, in the phenomena of parasitism and symbiosis.

Moreover, it is evident that they take on a practical interest, for one can henceforth study in vitro the action of chemical compounds capable of retarding or preventing the development of the parasite.

(Experimental Tobacco Institute of Bergerac)