THE FIELD CULTURE OF ERGOT IN THE USSR

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The Field Culture of Ergot in the USSR

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Alkaloids of ergot, or ergoalkaloids, are widely used in medicine. In pure form and also in the form of hydrogenated derivatives and in combination with other therapeutic agents, they are used in obstetrics, in diseases of the blood system, in nervous disorders, and in several other ailments (1, 2).

The raw material base for the production of the ergoalkaloids are the sclerotia (algaroba) of the fungus Claviceps purpurea, Tul., which is parasitic to rye, wheat, barley, and other grass and sedge plants.

The requirements of the pharmaceutical industry for ergot was practically covered by the collection of the sclerotia on rye plantings in the process of harvesting and screening the grain. In recent years, however, a decrease has been observed in the ergot supply, caused by a decrease in the affliction of rye plantings by this parasitic fungus as a result of the improvement of the agricultural techniques.

In the USSR ergot is found in all of the plant-life zones, except the deserts and tundra; favorable conditions for its mass growth are created with a raised relative humidity (70 % and more) and a moderate temperature (12-15°C) in the germination period of the sclerotia that have overwintered and during the flowering of the rye. Such conditions occur most frequently in the West and Northwest of the non-black earth zone of the USSR (3).

The negative aspect of the utilization of native ergot for the production of alkaloids is the sharp fluctuation of its productivity from year to year in relation to the yearly conditions and the uncertainty of the quantitative and qualitative composition of the alkaloids in it. There have frequently been cases when large consignments of prepared ergot contained no alkaloids at all.

It is incomparably more convenient to use sclerotia of ergot produced by means of artificial inoculation of rye with special strains of the fungus.

In this case it is possible to obtain sclerotia with an alkaloid content of 0.3-0.4 % and higher, whereas in the preparation and use of wild-growing ergot the sclerotia are received with an alkaloid content of 0.05 % (State pharmacopeia of the USSR, Izd. VIII; OST NKVT 6632/222). Meanwhile, the industrial processing of ergot containing insufficient alkaloid requires significantly larger losses of labor, time and materials than that required for ergot rich in alkaloids.

The necessity for an artificial culture of ergot on rye developed first in the West European countries, which were using and depending on imported ergot. At present, significant successes have been achieved in this field, particularly in the Czechoslovakian Republic and the Hungarian People's Republic (4, 5).

In the USSR, a research project on the field culture of ergot was conducted by VILAR (All-Union Institute of Medicinal and Aromatic Herbs) and its experimental stations (6 et al.). Before entering into the production utilization of this method a whole series of questions had to be worked out: to find simpler and more effective procedures for the inoculation of the rye with the ergot; to determine the regions of cultivation; to define more accurately the effect of the geographical position and the species of the host-plant on the alkaloid content of the sclerotia. What impressed most of all, because of the simplicity and ease, was the inoculation of the rye by spraying with a suspension of spores at the period of flowering, which in essence was an imitation of the natural process of infection.

The experiments that were conducted at many points in the USSR, which differ in natural conditions, and having variants in the time of spraying, composition of the suspension, and age of the spores, gave no positive results. The yield of sclerotia in all cases remained insignificant, only slightly exceeding that of the control.

The second inoculation method, which was investigated in detail by VILAR, consisted of the creation of concentrated nidi of the infection by means of introducing the sclerotia of ergot on a sowing of rye. The observations disclosed the interesting fact that the germination of the sclerotia in the majority of cases does not coincide in time with the period of the rye's flowering and therefore it cannot guarantee an increase of the infection.

A sure method for the artificial inoculation of rye with ergot is the active injection of the infection into the flower with needles wetted in a suspension of spores. This method is unaffected or little affected by the annual conditions and was used by us for the production inoculation.

It was very important to discover how the cultivation in the different geographical zones would affect the alkaloid content of the ergot; it is known that in the preparation of ergot sclerotia in the North and Northwestern Oblast of the European sector of the USSR and in Siberia they very often contain no alkaloids. With this purpose the rye in different points of the country was inoculated with a high-alkaloid strain of ergot. The results of the experiment are presented in table 1.

As seen from table 1 the ergot strains retain the ability to actively produce alkaloids regardless of the region of cultivation. Because winter rye is

The resultant strain of the fungus goes through a preliminary field evaluation on VILAR's experimental plot, where they determine the ability of the strain to infect rye (virulence), its yield capacity and alkaloid content.

Also an important indicator of a strain's production usefulness is its stability in regard to the alkaloid content. Table 3 gives a representation of the effect of selection on the alkaloid content and stability of a strain.

The maternal material, in the form of a test-tube pure culture, is transferred by VILAR to the Lekrastrest (Medicinal Herb Trust) sovkhozes engaged in the growth of ergot. Further reproduction of the strain is made in the sovkhoz laboratories by the VILAR method. The growth of the fungus culture for the infection of the rye is conducted at a temperature of + 18-20°C on beerwort-agar in flask-matrasses with a calculation of 6-8 flasks of the culture to one hectare (ha.) of rye.

A culture older than a month is suitable for use. In a dark, cool place the culture retains its viability for a period of 3-4 months.

The inoculation of the rye plantings with the fungus is the crucial moment in the culture of ergot. For this, in the Lekrastrest sovkhozes, they use hand boards consisting of a piercing board (5 needles per cm^2) and a press board, on which is attached a piece of felt that has been saturated with the suspension of spores.

The process of infestation consists of clamping the heads of rye between the needles and the felt, as a result of which the spores of the fungus are introduced into the pierced flowers. The efficacy of the inoculation depends on the timeliness of this operation. Special experiments and production observations showed that the best time for the inoculation is the beginning of the rye's heading, when the awns in 50-60 % of the plants have emerged from the tubes, and for a period of 3-4 days after this. The inoculation must be made in the cool period of the day, best in the second half of the day and in the evening.

Maturity of the ergot sclerotia occurs within 5-6 days after inoculation; this coincides with the wax ripeness of the rye's grain. The ripe sclerotia have a characteristic brownish-violet color; they are hard to the touch and non-adhesive. In this period they contain the largest amounts of alkaloids (fig 2).

The harvest of the ergot presents great difficulties. The sclerotia ripen at different times and are prone to fall off; therefore on small plots, where the collection of the sclerotia is done manually, it should be made periodically after 4-5 days until the harvesting of the rye for the grain. On the large production sowings the rye inoculated with the ergot is harvested, using a combine with a high cut, during the wax-ripeness phase. In this case the sclerotia are separated from the grain with the assistance of concentrated salt solutions, utilizing the different specific weights of the grain and the ergot sclerotia, or on complicated grain-cleaning machines.

The farms conducting the cultivation of ergot are in need of drying equipment with a large capacity because the grain and sclerotia coming from a combine

not grown everywhere, it was expedient to discover how a strain of the fungus would react if cultivated on other species of plants.

The results of the experiments with strain No. 180 are presented in table 2.

It follows from table 2 that the strain of the fungus stably maintains its alkaloid content on the different species of plants of the grass family. The small fluctuations of the alkaloid content is completely contained in the range of variation observable in the inoculation of winter rye with this strain and therefore cannot be evaluated as the result of an effect of the host-plant on the fungus. This makes it possible to recommend that ergot be cultivated on spring rye in those regions where winter rye is not grown. The data in the literature also indicate the possibility of producing good yields of ergot sclerotia on sp ring rye.

Great attention was paid also to the clarification of the agricultural engineering requirements for rye plantings that are intended for the growth of ergot, to the establishment of the best phenological periods for the inoculation of the rye with the ergot and the other conditions of cultivation (fig 1).

All of these investigations offered a theoretical basis on which to lay the foundation of production appropriation for a new medicinal culture.

The whole process of cultivation of a field culture of ergot is divided into the following stages: 1) selection of the alkaloid strains; 2) growth of the infectious material; 3) inoculation of the rye with the fungus culture; 4) harvest of the ergot sclerotia.

The first two processes are carried out under conditions of strict sterility and is, in actuality, microbiological work.

Selection of the strains is accomplished by VILAR. The necessity for selection has been brought about by the fact that unsorted natural ergot very often contains little alkaloid and its use for the production of the infectious material is economically unfeasible.

For the production of an ergot strain with the required alkaloid content of the desired chemical composition of the alkaloids, VILAR utilizes the widely used medthod of halves. In substance it is the grinding of half of a sclerotium and the analysis of the resultant powder. The quantitative evaluation of the alkaloid content is geven according to Rumpel (7); the qualitative characteristics are evaluated according to the paper-chromatographic method according to Macek (8).

If the content and composition of the alkaloids correspond to the requirements that have been established, the second half of the sclerotium is used for the production of a pure culture of the fungus. At VILAR, yearly, several thousand of such individual analyses of sclerotia are made, with innumerable seedings of the pure cultures on nutrient media.

contain much moisture and require immediate drying. In the heating of the grain and a mustiness of the sclerotia the alkaloid content is reduced sharply.

The yield of ergot depends first of all on the quality of the inoculation and the condition of the rye planting. A high yield of sclerotia can be obtained only on plots of rye where the biological yield of grain is no less than 20 centners/ha.

The growth and development of ergot requires an abundant nitrogen supply; therefore the planting of rye designated for the production of ergot receives one or two additional nitrogen feedings in the period of vegetation in addition to the primary manure and mineral fertilizers.

By observation of the rules for cultivation the average yield of ergot sclerotia on the VILAR experimental plots in Moscow Oblast amounted to 269 kg/ha. in 1958, and for isolated strains - up to 467 kg/ha. In a Moshkovo sovkhoz (Novosibirsk Oblast) the biological yield in 1958 was established at 232 kg/ha. In machine harvesting, the storehouse yield of sclerotia is significantly less than that of the biological because in the simultaneous harvesting in the phase of wax ripeness many of the sclerotia are far from attaining normal size.

At the present time the Lekrastrest sovkhozes have mastered the field culture of ergot and issues a raw material of high quality, although a whole series of problems require solution. These are for the most part related to the mechanization of the processes of inoculation and harvest.

Beginning with 1959 VILAR has begun to issue monoalkaloid strains of ergot, which produce only one of the alkaloids from the ergotamine or ergotoxine group.

The introduction of these strains into production gives a possibility of assuring the pharmaceutical industry a raw material that will significantly simplify the reprocessing and production of pure ergoalkaloids.

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Table 1

The alkaloid content of ergot sclerotia growing in the various regions of the USSR.

Point	Alkaloid Content (in percentage) 1956	1957	1958
VILAR (Moscow Oblast).	0.38-0.67	0.59-0.65	0.33-0.53
Moshkovo sovkhoz of the Lekrastrest (Novosibirsk Oblast).	0.37	0.58	0.49
Okunevskiy sovkhoz of the Lekrastrest (Kirov Oblast).	0.39	0.63	0.40
B. Mozheykovskiy sovkhoz of the Lekrastrest (Grodno Oblast).	0.40	-	0.39

Table 2

The effect of the host-plant on the production of alkaloids by ergot (VILAR, 1957).

Species of plant	Alkaloids content in the sclerotia (in percentage)
Winter rye	0.550-0.620
Spring rye	0.568
Kupriyanov rye	0.630
Pyreyno-wheat hybrid	0.522
Regneriya	0.600
Awnless bromegrass	0.537
Chewing fescue (F. rubra)	0.552
Dew grass (D. glomerata)	0.586

Table 3 and figs. 1 and 2 are not available to the translator.