STUDIES REFERRING TO METHODS OF SUNFLOWER ARTIFICIAL INOCULATION WITH SOME PATHOGENS

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SUMMARY

There are presented the standard methods of sunflower artificial inoculation with Plasmopara helianthi, Sclerotinia sclerotiorum, Phomopsis helianthi, Orobanche sp., in view of getting knowledge about the cvars. behaviour or of establishing the efficacy and proper time of chemical treatment application.
As a conclusion it is considered that for a method to be commonly accepted it has to meet the following requirements :
- to reproduce as true as possible the natural phenomenon
- to be easily applied
- under the same conditions to get similar results.

INTRODUCTION

Sunflower is one of the most susceptible crops to the attack of some pathogens included in almost all systematic groups.
The modern methods of integrated control include the use of both resistant cvars. and hybrids and some chemical treatments applied on seed or in vegetation. To establish these factors it is necessary to use artificial inoculation methods which enable to know the cultivars behaviour but also the chemicals efficacy.

MATERIALS AND METHODS

To achieve the infections by artificial inoculation it is very important to take into account the following :
- the age of both the host and the pathogen in the moment of impact between the two organisms ;
- inoculum type used provided the organ by which the pathogens can become naturally infectious ;
- conditions of humidity, temperature and sometimes light, conditions which depend directly on the pathogen biology.

RESULTS AND DISCUSSIONS

Methods of artificial inoculation with Plasmopara helianthi.

As the pathogen Plasmopara helianthi is oospores soilborne or which are in/on vegetal debris, their spreading being assured by zoosporanges, they can be used in artificial inoculation with both propagule types.

To get a heavily infected plot (with oospores) it is required a sunflower monocropping, for 5-6 years.

Such a field could be used mainly to check out the efficacy of some antiperonosporic chemicals applied to treat sunflower seeds in view of preventing and controlling Plasmopara attack.

Other artificial inoculation methods consist in :

- spraying a zoosporanges suspension on germinated seeds (before planting) or on plantlets in phase of cotyledons - first pair of true leaves ;
- maintaining germinated seeds (length 0.5-1 cm) for 6-10 hours in a zoosporanges suspension, at 16-18°C.

A very important element to get successful artificial inoculations is the concentration of zoosporanges suspensions. Novotelnova (1966) thinks that a great quantity of zoosporanges in suspension could be a limiting element in their germination. Goossen and Sackston (1968) got infections with a concentration of 100 spores/ml.

To better know the individual behaviour of every sunflower plant in view of its selection as a source of resistance gene, the most adequate method would be the immersion of germinated seeds in zoosporanges suspension (Table 1).

- Table 1 - Percentage of sunflower plants with downy mildew symptoms obtained by using various artificial inoculation methods

Method	Variant	Infected plants (%)				
		Record	HS 52	HS 90	S 1358	Check
Oospores in soil		61.5	79.3	83.0	91.3	78.7
Spraying a zoosporanges suspension on :	germinated seed	69.3	73.4	85.1	76.0	75.9
	cotyledons	56.3	36.4	75.3	63.9	58.0
Immersion of germinated seeds in zoosporanges suspension		96.3	97.3	100	100	98.4

Methods of sunflower artificial inoculation with Sclerotinia sclerotiorum.

Sclerotinia sclerotiorum is sclerotia and resistance mycelium borne and it overwinters on vegetal debris. Its spreading is assured by ascospores produced in apothecia formed on mature sclerotia and by mycelium segments transported by insects or wind.
In case of sunflower artificial inoculation with Sclerotinia sclerotiorum the following methods can be used :

- mycelium grown on PDA and introduced by incision into stalk or head ;
- mycelium grown on oat seeds introduced by incision or placed, without any incision, on the leaf axille or under one of the involucrum bracte. In all case humidity is aasured by a water soaked cotton plug ;
- use as inoculum of a mixture of mature sclerotia, ground with sand 1/19 (v/v) and placed into soil by dibble at pocket, when planting.

From Table 2, it appears that the most severe artificial inoculation method with Sclerotinia sclerotiorum is to place the pathogen as vegetative form in direct contact with the host tissues (Iliescu and Pîrvu, 1977). It is also to mention that the use of the method does not consider at all the mechanical resistance of the host, that being defended only by the biochemical resistence (Pustovoit and Krasnocutskaia, 1976).

If it is meant to use as inoculum, ascospores, they can be obtained on mature sclerotia placed in moist chambers, at 14-16°C, and light of 40,000 - 60,000 lucs.

Following the obtainance of apothecia with asci and ascospores spreading, the latter are traped on filter paper covering the inside wall of the moist chamber.

Artificial inoculation in thus obtained only by application on leaves, stalks or heads of a segment of this filter paper charged with ascospores, as soon as a high moisture (>90%) was obtained and maintained for 48 hours.

- Table 2 - Efficacy of some methods of sunflower artificial inoculation with Sclerotinia sclerotiorum function of the inoculum type.

Inoculum	Frequency infected plants			
	4-6 leaf pairs	flower button	post flowering	head
Mycelium by incision	98.3	96.3	loo	loo
Mycelium without incision	73.5	75.4	83.4	9o.1
Mixture sclerotia + sand	85.3	81.3	81.9	8o.6
Ascospores on filter paper	91.4	89.3	83.3	8o.3
Ascospores suspension	64.5	54.1	69.4	91.3

Methods of sunflower artificial inoculation with Phomopsis helianthi.

There has not been established a general accepted artificial inoculation method for this pathogen, so far.

Based on the research works carried out in France (Peres and Regnault, 1986; Bertrand and Tourvieille,1987 and Tourvieille and Pelletier,1988) plants artificial inoculation is recommended (plant segments) with/without injury by ascospores suspension spreading (various concentrations).

Our works were meant to get the inoculum both as ascospores in quite sufficient quantities to make the suspensions, and as mycelium.

From naturally infected plants, the parasite is isolated and purified on PDA supplied with sunflower stalks extract. On this substratum are placed wooden toothpicks used for artificial inoculation which are colonized by the pathogen in 2-3 weeks. To get ascospores, stalks segments showing typical symptoms and maintained under natural conditions untill Spring, are placed in moist chambers, at 23-24°C, in a thermostat with artificial light.

Six days later, mass perithecia occurrence can be observed. The segments with perithecia are placed in distilled water flasks shaken for 5 minutes to release asci and ascospores. After sieving the suspension is ultrasoned to release ascospores and it can be preserved up to 2 days, at 4°C (the temperature does not allow spores germination thus preserving their viability).

The suspension thus obtained adjusted to a titre of 5×10^3 ascospores/ml is atomized on the whole plant ensuring a relative humidity of 95-100% for at least 48 hours.

By suspension atomization can also be inoculated leaves (mainly at their ends), petioles or stalks and in that case humidity can be maintained by coating with tinfoil of the inoculated part of the plant. A higher percentage of infected plants or plant segments can be obtained by making incisions (petiole, stalk), injury of lamina with carborundum or by direct insertion into tissues to toothpicks colonized with mycelium.

As in case of inoculation with Sclerotinia sclerotiorum we do not recommend injury as it removes the mechanical resistance given by the tegument integrity.

Encouraging results were obtained by Peres and Regnault (1986) by simply placing the contaminated stalk segments, bearing perithecia at the level of soil or at various hights, close to sunflower plants. The differences of attack frequency registered in the former case (10%) compared with the latter (40%) are justified by the spreading weakness of ascospores when leaving the asci. (Masirević, personal communication).

There is no inoculation methodology so far for sunflower seeds with Diaporthe-Phomopsis although the geographical distribution of the parasite can be explained only by handling the contaminated breeding material.

Methods of sunflower artificial inoculation with Orobanche spp.

One of the anthophytoses which causes great problems for sunflower, tobacco and tomato crops is caused by the parasite phanerogama Orobanche spp. For inoculation, are used seeds of the parasite collected in the previous year from mature pods and kept during winter under natural conditions or in the refrigerator.

The inoculum is a mixture of sand and seeds 19 : 1 (v/v) out of which 5-8 gr are placed in each nest (pocket) with the dibble, when planting.

CONCLUSIONS

In order that an artificial inoculation method be used successfully the following requirements are to be fulfilled:

- to be easily applied thus enabling screening of a great number of forms or chemicals/rates;

- to enable under the same application condition to get reproductible results ;
- the artificial inoculation technique be close as much as possible to the natural phenomenon ;
- to enable to get some differenciated results on account of the genetic nature of the biological material to be tested and of the rates, too, in case of checking out the efficacy of some chemicals of fungicide or fungistatic activity.

REFERENCES

BERTRAND F., TOURVIEILLE D.,1987. Phomopsis tournesol : Tests de selection.

GOOSSEN P.G., SACKSTON W.E., 1968. Transmision and biology of sunflower downy mildew. Cand. J.Bot. 46(1) : 5-18.

ILIESCU H., PIRVU N., 1977. Contribuţii la studiul metodelor de infecţie artificială folosite în ameliorarea rezistenţei florii-soarelui la boli. Probleme de Protecţia Plantelor 5 (4) : 407-422.

NOVOTELNOVA N.S., 1986. Lojania mucinistaia rasa podsolnecinika. Ed. Acad. Nauk SSSR, 149 p.

PÉRÈS A., REGNAULT Y., 1986. Phomopsis helianthi : Production d'inoculum et mise au point d'une méthode de contamination artificielle. Information Techniques CETIOM 95 (2) : 24

PUSTOVOIT G.V., KRASNOCUTSKAIA O.N., 1976. Wild species of Helianthus as initial forms in sunflower breeding for imunity. "7th Inter. Sunf. Conf. Krasnodar " : Vol.I : 402-408.

TOURVIEILLE D., PELLETIER C., 1988. Jugement de la résistance du tournesol au Phomopsis sans tunnel en filet avec humectation controlée. Information Techniques CETIOM 103(2): 28-29.