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TUBERCULINA PERSICINA (DESM.) SACC., AS A HYPERPARASITE OF GYMNOSPORANGIUM FUSCUM DC

TUBERCULINA PERSICINA (DESM.) SACC. , KAO HIPERPARAZIT NA GYMNOSPORANGIUM FUSCUM DC

Abstract

The data on frequency and significance of occurnence of *Tuberculina persicina* (Desm.) Sacc. as a hyperparasite on *Gymnosporangium fuscum* DC, the development of associate fungi, the possibility of culture of hyperparasite on artificial media, have been investigated. The possibility of primary parasite control, time of application of hyperparasite and its efficiency have been studied by the artificial inoculations. The perspectives of biological control of the pathogene responsible for the pear rust have been discused, and the need for additional investigations indicated.

Key words: *Gymnosporangium fuscum, Tuberculina persicina,* hyperparasites, biological control.

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Izvod

Dati su podaci o učestalosti i značaju pojave *Tuberculina persicina* kao hiperparazita na *Gymnosporangium fuscum*; ispitivan je paralelni razvoj združenih gljiva i karakter tog odnosa; provjeravana je mogućnost uzgoja hiperparazita na vještačkim podlogama. Mogućnost suzbijanja primarnog parazita, vrijeme primjene hiperparazita i njegova efikasnost ispitivani su vještačkim inokulacijama. Razmatrane su perspektive biološke borbe protiv prouzrokovača rđe na kruški i ukazano je na potrebu dopunskih istraživanja.

Ključne riječi: *Gymnosporangium fuscum*, *Tuberculina persicina*, hiperparaziti, biološka borba.

INTRODUCTION

In last thirty years the attention paid to biological control of some plant diseases has been increased, but still one cannot talk about the decisive advancement which would essentially replace the classical methods of plant parasite control. Antagonistic relationship of fungi occuring on higher plants is less evident than among the plant pests, especially insects, but it does not mean that this relationship should be neglected, on the contrary. Nowdays, when the environmental protection gains on significance, where the pesticides are observed as great pollulants, it is necessary to search for the alternative solutions, based on biological control.

Long ago, the cases of association of some fungi and their antagonistic relations were designated either as anabiosis, hyperparasitisme of competition for the substrate. Only a few out of some 200 genera and almost 500 species of hyperparasites and their relations with primary plant parasites have been studied in more details (K r a n z, 1981).

Until now, the antagonistis of fungi the most studied are the species of genus *Trichoderma*. Some other species, especially from genus *Eudarluca (Darluca), Tuberculina, Cicinobulus, Verticillium, Ampelomyces, Penicillium, Aspergilus, Gliocladium, Coniothyrium*, (T a r u n i n a, 1983) were also investigated. Number of those for which the biological preparations for the control of phytopathogenic fungi have been obtained is very low. In Sweden, for instance, the preparation as tabletted biomass of *Trichoderma viride* has been patented. Out of the same fungus, preparations have

also been produced in Russia, and the procedure of massive production of *Ampelomyces cesati* has also been elaborated. In the USA a granulated biopreparation on basis of *Trichoderma harzianum* was produced, while in England a biopreparation based on *Peniphora gigantea* is being applied. A biopreparation consisting of pycidia of *Coniothyrium ministans* - for control of onion white rottening, and a protected fungicid product consissting of spores and mycelim of immunizing comansals (*T. polioporum, T. viride* and *Scytallidium lignicola*) have been elaborated (T a r u n i n a, 1983).

Larger application of antagonists in the agricultural practice is limited by insufficient level of knowledge of majority of antagonistic species, and their development requirements in relation to the primary parasites.

In Montenegro, there has been no more profound, systematic work on these problems, although some former researchers and ourselfs, we have recorded the occurrence, of certain number of associated fungi especially on phylloplane, and their antagonistic relations (M i j u š k o v i ć, 1976; Mi j u š k o v i ć & V u č i n i ć, 1974; 1982). On the basis of these researches, it was established that, among a great number of recorded cases of associated fungi, *Tuberculina persicina* is one of the most significant hyperparasites on *Gymnosporangium fuscum*, causal agent of the pear rust. The researches of this particulary case have been realisated in Agricultural institute of Podgorica (Montenegro) in 1988-1992, since this hyperparasite relation has not been sufficiently studied in the world either.

OBJECTIVES AND METHODS OF WORK

In order to realize better the significance of *Tuberculina persicina* as a hyperparasite of *Gymnosporangium fuscum* one should, first, establish the importance (in given ecological conditions of the south part of Montenegro) of *G. fuscum* occurrence and the demages on peras that may be cased by this parasite; on the other side, the appearance and life cycle of *T. persicina*, biological and ecological requirements of the both fungi species have to be studied, as well as the coincidence of their developmental stages, time and manner of dissemination, the kind of antagonistic behavior and the consequences for the primary parasite. In that way it would be possible to get a view of their reciprocal relations in natural conditions. Possibility of hyperparasite cultivation on artificial media, the

way and time of artificial inoculations, the level of efficiency of antagonistic species on the adventual decrease of damages that the pathogene may cause on pear, decrease of the infective potencial in the orchard, and particulary, possibility of practical use of the hyperparasite in biological control against the phytopathogenous organisms - represented the further purposes of the performed investigations.

The occurrence of the both fungi species, their distribution, frequency and biological cycle were monitored parallely in natural environment in several pear orchards on the Montenegrin Sea cost (Herceg Novi, Bar), in Podgorica and Nikšić. In 1989 in Bar the evaluations were done on June 20 and August 31. On four cultivars (William's, Trevush, July's early and an unknown one) 300 leaves taken from all the sides of fruit tree crown were examined and the number of leaves infected by *G. fuscum* was recorded. By examination of 300 spots on infested leaves, the occurrence of hyperparasite was checked. The same examination was done the next year, 1990, in the orchard in Bar.

The spore germination of *T. persicina* has been studied before the investigation of the possibility for growing this fungus on artificial media. It has been done on room temperature (23 °C - 30 °C) and in termostate on 25° and 28 °C, in hanging drop and in spore suspension in destilated water poured out in a thiny stratum over the glass plate. This has been performed in three repetitions, and evaluation of germination has been done after 3, 5 and 24 hours.

Cultivation on artificial media, after unsuccesful culturing on some classic one, has been attempted on the following: carrot and potatos plugs, yeast-extract agar, PDA-Difco, agar water, V-8 agar. Sporodochia of *T. persicina* from naturally infected pear rust spots, taken from Herceg Novi and later from Podgorica have been used as the source of inoculum. The cultivation has been done in test tubes and Petri-dishes, at 24-25 °C, in darkness and on the light. Sporodochia were transfered to the media directly, as well as spores, whose germinating capacity had been previosly checked.

The artificial inoculations of *G. fuscum* stromata (spermagonia) were performed on naturaly infected pear leaves: by suspension of *T. persicina* spores in water and by direct transfer of spores to *G. fuscum* stromata, separatley from the upper and lower surface, using a needle or a camel-hair brush.

In order to avoid the possibility of unintentional infection of *G. fuscum* stromata, later on (1991, 1992) the pear leaves were previously artificialy inoculated by *G. fuscum* basidiospores, and after that, during the development of the fungus, using the method described above, the artificial inoculations have been performed by hyperparasite spores, starting with the appearence of *G. fuscum* spermatia.

The inoculated leaves have been isolated by plastic bags. To maintain the air humidity, moist absorbent cotton has been inserted inside the bags; they were taken off two days later.

RESULTS OF THE RESEARCHES

Significance of *G. fuscum* as pear parasite

Gymnosporangium fuscum as pear parasite was recorded in Montenegro back in 1949 (M i j u š k o v i ć, 1950). This parasite was a subject of detailed studies in period after 1971 (M i j u š k o v i ć, 1976; 1992). It was established to be, certainly, economically the most significant species of this genus in Montenegro, but also in some other parts of ex Yugoslavia, especially those with mediterranean climate. Aecial stage develops in form of orange spots on the leaves, rarely on the branches and fruits. Stromata are formed, at first with pycnidia on the upper surface of leaves, and, later, with aecia on the lower surface. In favorable conditions the spots may cover almost the entire leaf and cause its premature dropping. (Fig. 1).

Development of *G. fuscum* aecial stage on the pears is sufficiently known. It lasts long, from the beginning of May until October-November. After the occurrence of yelloworange pustules with pycnidia (Fig. 2), originated since the end of June, development of brown stromas begin on the undersurface of leaves. Later, they alter to wartlike forms with sprouting of peridium and developing roestelia type of aecia in fall (end of September or beginning of October) and dissemination of aeciospores from mid October (Fig. 3).

Originally described from *Juniperus sabina*, teliostage of this fungus on Montenegrin coast, where *J. sabina* does not exist, develops only on *Juniperus oxycedrus* and *J. phoenicea*. Maturation of teliosori on juniper-trees takes place at the end of March or the beginning of April, and pears infections occur from the foliation. Incubation usually lasts 10-20 days,

so the first spots on the leaves occur from the end of April, most frequently in the first decade of May.

The level of infections of pears with *G. fuscum* greatly depends upon the vicinity of orchards to the uncultivated lands on which the two mentioned *Juniperus* species spontaneously grow. Infection rate also depends upon the climatic conditions existing in period of dissemination of basidiospores. Du to mediterranean or modified mediterranean climate in the areas of pear rust occurence, the rains, which are quite frequent at that time, usually make possible first the gellefication of teliosori, and consequently infection with basidiospores.

The transmition of fungus from pear (by aeciospores) to *Juniperus* occurs in fall, in favourable weather conditions, when rainy period starts again. Dissemination of aeciospores lasts longer than the basidiospores release.

This short survey of *G. fuscum* development was necessary for easier understanding the occurrence and development of *T. persicina.*

DEVELOPMENT OF T. PERSICINA (DITM). SACC.

T. persicina as hyperparasite upon *G. fuscum* was recorded in Montenegro back in May 1972 in Herceg Novi (M i j u š k o v i ć, V u č i n i ć, 1974). Rate of spots of *G. fuscum* on pear leaves on which *T. persicina* was recorded, was 83,9% in 1972 and 93% in 1973. In subsequent years the parasite used to be found in large numbers, but the infection rate had not been numerically checked until the beginning of this project.

T. persicina was, for the first time, recorded and described by D i t m a r (1817) in aecia of *Circea lutetiana*. L e v e l y e (1848, cit. according to V l a d i m i r s k a y a, 1939) recorded this hyperpastie in aecia of rust in a number of plants. *T. persicina* description was made by G o b i (1885; 1886). He erroneously listed this fungus in family *Ustilaginales* under the name *Cordalia persicina* Gobi. Abreast with the explanations on some biological traits, the author points that *Cordalia* differs from the typical *Tilletiaceae* because its spores are not transformed into powdery mass. The fungus sporulates only in the conditions of increased moisture, while in arid and warm period sclerotia are formed. S a p p i n - T r o u f f y (1896-1897) discovered that *T. persicina* directs the stems of its hyphs to

the host's cells of phytoparasite, feeding itself on its protoplasma and causing cells' dying out. This author did not note formation of sclerotia. More recently this fungus has been listed into *Fungi imperfecti,* fam. T*uberculariacae* (Syn. : *Uredinula persicina* Spegazzini, 1880; *Tubercularia persicina* Ditmar, 1817; *Cordalia persicina* Gobi, 1885).

Occurence of *T. persicina* was recorded by many authors in aecia of species *Puccinia, Gymnosporangium, Uromyces, Melampsora, Coleosporium, Phragmidium.* It is interesting to note that we have been finding it exclusively on aecia of *Gymnosporangium fuscum* in southern part of Montenegro and only once on *Gymnosporangium tremelloides* in northern region.

V l a d i m i r s k a y a (1938) reports that *T. persicina* is distributed from the very north of Europe to the south of Africa. For its narrow specialisation as a hyperparasite, it might be interesting for the control of rusts.

In our studies *T. persicina* occured early, already in stage of spermagonia of *Gymnosporangium fuscum*. Its earliest record was made in mid May and more frequently at the beginning of June. *T. persicina* is widely spread in pear orchards infected by rust by the end of June.

The hyperparasite, according to our results, inoculates zones with spermatia in the frame of orange spots on the leaves, leaf stems (sometimes also sprouts) of pear, both from the upper and the lower surface of leaf, where, on the later, it reaches almost the entire surface area which corresponds to spermatia. It occurs as sporodochia (Fig. 4), which sprout by tearing the epidermis (Fig. 5). On that occasion, the individually sporodochia ling and cover almost completely the part of the spot which fits to the position of spermatia. Macroscopically observed, sporodochia are violet, violetreddish, violetbrownish or greyish, depending upon the age and quantity of unscattered conidia.

Conidiophores, in form of dense bundles radially distributed, observed isolated have a pale-violet or palebrown color. They are simple or ramified on the summit, sometimes rounded or englarged, sometimes slightly cogged, nonsepted. Their dimensions range from 43-56 x 3,3-5,2 μm (average 47,8 x 3,7 μm). Conidia are usually spheric, weakly coloured by bright nuances of violet, pink or brown. Their size ranges from 6,6-10,5 x 4,9-9,9 μm, in average 9,16 x 7,9 μm.

DEMONSTRATION OF ANTAGONISTIC RELATIONS

Occuring early and developing on the spermagonia stage of *G. fuscum,* hyperparasite *T. persicina* prevents increase of stromata of phytopathogene on the leaves and evolution of *G. fuscum* from spermogonia to aecia stage, stopping thus the development cycle of the parasite. Gradually penetrating all the tissues, it completely removes and replaces *G. fuscum* (Fig. 6), producing finally a type of sclerotia, which, later on, may drop from the leaf tissue or stay on the leaf, and in its remnants winter on the ground (Fig. 7).

Significance of *T. persicina* as hyperparasite of *G. fuscum* is great, and it might be decisive if teliostage on *Juniperus,* especially on *J. phoenicea,* would not be regenerated from the mycellia in infected branches for a number of subsequent years.

OCCURRENCE AND DEVELOPMENT OF T. PERSICINA IN NATURAL ENVIRONMENTAL CONDITIONS

In natural conditions *T. persicina* appears shortly after the occurence of spots on pear leaves and formation of *G. fuscum* spermagonia. The period of infection by hyperparasite is linked to duration of that stage respectively of duration of exudate on the surface of spots and spermagonia, what amounts about a month. All *G. fuscum* spots on pear leaves do not appear, namely, at the same time and their development and spermagonia number increase as the time goes on. In 1988 in Herceg Novi, where the *G. fuscum* infection of pear was severe, at the beginning of July it was found that stromata produced by fungus on the leaves were practically 100% reached by hyperparasite. Out of 161 leaves examined, 85 had the orange spots of rust (1-9 spots per one leaf, respectively 209 spots), and each of them had the hyperparasite *T. persicina* as well. In other places both rust and hyperparasite occurrence have been rarer. More intense occurrence of rust and its hyperparasite in Herceg Novi than in other localities are related to the immediate vicinity of the hill abundant with juniper trees, and the vicinity of the sea, as well as, to some extent, to moister of soils.

First occurrence of *T. persicina* in 1989 was recorded in the mid May (spring started somewhat earlier) and the infections were continued for a month. The level of attack of *G. fuscum* on one and a parallel *T. persicina*

Tab. 1 — Occurrence of *Gymnosporangium fuscum* and its hyperparasite *Tuberculina persicina* on the pear leaves in 1989

Site	Date of examination	Cultivar	Infection of leaves by *G. fuscum* %	Number of spots per leaf (from -to)	Average of spots per leaf (for all leaves	Average spots on infected leaves	Percentage of spots with hyper-parasite *T. persicina*	Development stages of *T. persicina* Sporu-lation %	Sclero-tia %
Bar	6/22/89	Viljamovka	55	1 — 7	0,987	1,794	14,54		
	8/31/89	Viljamovka	83.6	1 — 12	1,716	2,100	54	53,91	46,09
	6/22/89	Trevuška	65.67	1 — 5	1,213	1,848	1,77		
	8/31/89	Trevuška	70.8	1 — 6	1,320	1,864	31,60	70,89	29,11
	6/22/89	July beauty	22.33	1 — 5	0,363	1,627	1,48		
	8/31/89	July beauty	29.20	1 — 7	0,484	1,658	3,60	88,80	11,20
Her. Novi	6/30/89	Unknown	76.40	1 — 8	1, 696	2,272	63,27		

Tab. 2 Attack rage of *Gymnosporangium fuscum* on pear leaves (300 leaves examined) in 1990

Site	Date of examination	Cultivar	Infected leaves %	No of spots per leaf (from - to)	Average spots per leaf (all leaves)	Average spots on infected leaves	Rate of spots (%) with hyperparasite *T. persicina*
Bar	7/12/90	Viljamovka	45,3	1 — 3	0,573	1,265	89,67
(Šušanj)		Trevuška	17,3	1 — 2	0,187	1,077	83,33
		July beauty	38,0	1 — 3	0,460	1,211	48,0

occurrence on the other side, were chacked that year in a orchard in Bar, on June 22, and August 31. At the end of August it was also monitored in how many cases *T. persicina* had still been in active sporulation phase, and what was the rate of sclerotia. The results of two examinations have been demonstrated in tab. 1. It is obvious that from one to the other examination the number of infected pear leaves increased to some extent, but the number of spots reached by hyperparasite *T. persicina* increased even more. In any case, in Bar, the conditions for occurrence of both fungi species were less favourable than in Herceg Novi.

The occurrence of primary parasite and hyperparasite in 1989 was also monitored in Sutorina (Herceg Novi) and in Nikšić.

High percentage of pear leaves have been infected (40,27%) in Sutorina, in young, a year before planted orchard but only 1% of these pear leaves had *T. persicina* sporodochia. On the old trees in the vicinity, 76,4% of leaves were infected and, 63,27% spots had hyperparasite. This difference may be explained by the lack of inoculum in the first case, as in the new orchard there has been no time to produce infective potential of *T. persicina* which would be maintained on dropped leaves in winter. Also, in this orchard, leaves dropped had been brought to the deeper soil strata by fall ploughing.

Natural infection of pear leaves by *G. fuscum* in Bar in 1990 (evaluation carried out the same way as in 1989) was demonstrated in Tab. 2. Infection by rust was lower, but the rate of spots with hyperparasite was significantly higher in 1990 (89,67%, 83,33%) than a year ago.

In order to examine more precisaly the conditions for hyperparasite development, implementation of the infection, lasting of the incubation and total active cycle, it was essential to suplement the investigations in nature by the artificial inoculations.

CULTIVATION OF T. PERSICINA ON ARTIFICIAL MEDIA

Sporodochia from natural infections of *T. persicina*, were used as inoculum. Cultivation has been done in test tubes and Petri dishes on temperature 24-25 °C, in darkness and on the light. Sporodochia were transferred to the media, as well as spores, whose germination capacity had been previously chacked.

Unfortunately, none of studied media (PDA, carrot plugs, potato plugs, yeast extract agar, PDA-Difco, agar water, V-8 agar) had proved to be favourable for *T. persicina* cultivation. Negative result has also been obtained when we attemped to transfer the sclerotia to nutritive substrates. The studies had been carried out in Phytopathological Laboratory of Agricultural Institute in Podgorica, as well as on Agricultural School of University in Novi Sad.

Streaking out the spore suspension with a wire loop onto the agar water plate resulted in rare spore germination. Their transfer to the other media did not produce the development of colonies. The same happend when 25% lacticacid has been added to agar water. Namely, forty-eight hours later, it could be clearly observed on microscope plates that some of the spores had germinated, but they did not produce the colonies development on the PDA-Difco even after 15-20 days.

Failure to obtain pure cultures on artificial substances of this very promisig hyperparasite has mainly interfered the work on artificial inoculations, and the examination of practical application of biological control. Finding the new madia remains to be a task for the further researchs.

ARTIFICIAL INOCULATIONS

Regardless of failure of culturing on artificial substrates, in order to examine the possibility of artificial inoculation of *Gymnosporangium fuscum* stromata on pear leaves by spores of *Tuberculina persicina,* five two-years pear plants, held over the winter in pots in a green hause, were moved on April 4, 1989 to the pear orchard in Bar, known by rust infection. The plants have been exposed to natural infections by *G. fuscum.* After the occurrence of first symptoms, the plants were returned to Podgorica and held in protected space.

Samples of *T. persicina* sporodochia, found in nature in Sutorina (Herceg Novi) on June 8 th, have served as a source of inoculum for the first artificial inoculations, in lack of cultures on the artificial substrates.

Inoculations were done in three terms: June 12 and 23, and July 3, 1989. The number of inoculated spots was limited since there were no more plants. Occurrence and the development of hyperparasite were monitored every day.

Inoculation by spore suspension has been done on 23 spots. The first occurrence of *T. persicina* on the lower side of spots was recorded 8 days later, on June 20. By the examination carried out on June 26, it was recorded that out of 23 aecial *G. fuscum* stromata, 21 was infected with *T. persicina* what makes 91.30%. There was no infection by hyperparasite on the noninoculated stromata.

In the second term (June 23) conidia of *T. persicina* were transferred directly to *G. fuscum* stromata from the upper surface of leaves. At that time the spermagonia were clearly visible, and the surface of spots were covered by exudate. The changes on stromata were recorded on July 3, certifing the success of the infection. On July 7, it was found that *G. fuscum* stromata from the upper surface mainly lost their common orange color, while on the undersurface in their frame young sporodochia of *T. persicina* occurred. Their development continues, and on July 17, it was estimated as very luxuriant. All the inoculations have been 100% sucessful.

New inoculations, performed on July 31, the same way as in previous case but only from the back of the spots, failed. For the infection, thus, it is necessary to have the spores of *T. persicina* on the upper surface of *G. fuscum* stromata until the spermagonia are covered by excudate (pycnial nectar).

Although the purple stocklets of sporebearing organs maintain themselves rather long, beginning og creation of sclerotia of *T. persicina* in some stromes infected on June 12, was recorded at the begining of August, but even in the middle of that month sporodochia with conidia still existed in some stromes.

As indicated above, infection of pears by rust and occurrence of hyperparasite were regularly more frequent at the seaside than in the hinterland.

In order to obtain a sufficient number of leaves reached by rust in Podgorica, where the Headquarters of the Institute are situated, and have the work be easier, it was decided to artificially infect the pear trees on the experimental field of the Institute in Podgorica (where there are no juniper trees), by teliospores of *G. fuscum,* and then to make the inoculation with hyperparasite. In Bioče, some fifteen kilometers away from Podgorica, where all the slopes of the hill are overgrown by *Juniperus oxycedrus*, on April 6, 1990 it was found that *G. fuscum* teliosori on them swelled up and gellyfied. The infecteted branches were brought to the laboratory, sori

were taken off and put into the distilled water; they were held in it until the teleospore start germinating. On 12 selected pear trees the branches with 10-15 leaves were labelled, and a day later (April 7) the inoculations were made by suspension of *G. fuscum* spores, applied by camel hair brush. The work was carried out on a rainy weather, when stomes on the leaves are opened, what offered better chances for having the infections. The branches with inoculated leaves were isolated by plastic bag, in a previously described way. In order to establish the occurances of the infection simptoms, inoculated branches were regularly checked. First such symptoms were recorded 17 days after the inoculation, on April 24. Small yellow spots on the pear leaves have, as the time was passing, been becoming better visible and larger. The success of the artificial inoculations by rust was extremely good. Pear leaves on May 3, were mainly covered by spots of *G. fuscum* (Fig. 8), what was even undesirable, as there was a chance for leaves to drop before the end of the trials with hyperparasite. The symptoms of infection were especially notable on the summits leaves which, in the moment of inoculation, were the youngest. At that time the occurrence of spermagonia has been noted. There were no natural infections on the experimental trees. By the examination on May 22, it was found that inoculated leaves were practically covered by stromata of *G. fuscum,* which meanwhile, increased in size; spermagonia were well visible and they started to change from orange to orangebrown color, which at some places was changing to black.

Sporodochia of *T. persicina*, obtained on pear leaves from Bar, have been used for the inoculum.

Suspension of spores of hyperparasite in distilled water was applied by a brush on the *G. fuscum* stromata from the upper surface of leaves, from the under surface and from the both sides. First inoculations were made on May 24, on two selected trees. Control on May 30, did not reveal the signs of T. *persicina* occurrence, while on June 7, the first occurrence of hyperparasite was rescorded.

Sporodochia obtained from these, first inoculations, have served as a sourse of inoculum for further infections. They were made on June 13, from the upper surface and from under surface of leaves. The first rare signs of infection appeared on June 26, but only on the leaves where inoculation was performed from the upper side of *G. fuscum* stromata, as in the case in previous artificial inoculations.

After the sporulation stage of *T. persicina* which in earliest infections lasted around a month, on the middle of July sclerotia formation started, but the sporulation process had not been completely stopped yet.

New inoculations were carried out on July 16. *T. persicina* spores from the natural infections in Bar have been used for inoculum. At that time, the stage of stroma - future aecia of *G. fuscum* had already started on the undersurface of leaves on noninoculated branches.

It is important to point out that the inoculations done on July 16, were not sucessful. At that time the stage of spermagonia in *G. fuscum* was ended; according to our investigations this stage is the only one which makes possible the hyperparasite development. In these cases, as well as in inoculations from the undersurface of leaf, *G. fuscum* continued its normal development. By a survey made on September 12, it was recorded that in that stromata of *G. fuscum* had been already formed on back of the leaves and some pseudoperidium started sprouting. Normal aecia have been formed later on. On the leaves where the infections by hyperparasite were sucessful, *G. fuscum* development was stopped in phase of hyperparasite penetration (Fig. 9). Stromata for future aecia were not being formed in these conditions, but whitin the earlier formed *G. fuscum* spots, sclerotia were produced, what was found by the survey carried out on August 10. Since the stage of spermagonia has already been completed on July, and extraordinary severely infected pear leaves with *G. fuscum* started to dry out, further inoculations were not done.

If the results from 1990 are compared to those from the previous year, it may be established that the effect of hyperparasite inoculation was similar, and mainly sucessful (Tab. 3).

Tab. 3. Results of artificial inoculations of *T. persicina* on *G. fuscum* in 1990.

Date of inoculation	Tree	Leaves number on branche	*G. fuscum* spots	Artifical inoculation Succseded Number	%	Failded Number	%
May 24	1/7	Twig broken	—	—	—	—	—
	2/12		29	29	100		
June 13	11/3	30	513	222	43	291	57
	10/4	9	21	13	62	8	38
June 13 (leaf back)	10/4 — 1* 11/3 — 1*	7	34	4	12	30	88
July 16	6/5	12	410	—	—	410	100
	9/6	15	470	—	—	470	100
Control	8	6	298	—	—	—	—

Lower rate of successful inoculations made on June 13 (from the upper surface of leaves) is probably conditioned by an unusually large number of infections of *G. fuscum* on one leaf preventing normal development of stromata of this fungus and occurrence of spermagonia.

In 1991 the experimental inoculations were repeated at first on the leaves of pear by teliospores of *G. fuscum* (April 16) and than by *T. persicina* spores (June 14, July 15). Unfortunately, artificial inoculations of *G. fuscum* have been so numerous that they have totally covered pear leaves and they dried and dropped before it was possible to make respective apprisial (Fig. 8).

Occurrence and the development of *T. persicina* hyperparasitic upon *G. fuscum*, both in natural infections and the artificial inoculations, have

* On leaves with the inoculation from the undersurface of leaf (10/4-1 and 11/3-1) it is possible that, in isolated bag, the inoculum has by chance passed to the leaves front.

shown that *T. persicina* is able to greatly influence the primary parasite development. *T. persicina* has proved as an efficient antagonist, favourable for biological control of pear rust. Culture of the hyperparasite on artificial media remains to be solved in order to obtain enough inoculum for pratical applications.

DISCUSSION

Although the occurrences of hyperparasite were recorded in last century, it is only now that this phenomenon is paid more attention to. It has been also stimulated by the increasing demand to protect the environment by replacing chemicals with biological control. For that reason B a k e r & C o o k (1974) in their book on biological control of plant pathogens could, justly, state this to be the first book completely devoted to biological control of plant parasites, the first publication detailed enough to justify this large field of research.

In the world, nowdays, special attention is paid to use of organisms (bacteria and fungi) as antagonists to pathogenes in the soil, but less on phylloplan. Biological control of pathogenes on the leaves has, during the recent years, been discussed in papers of a number of scientists; these papers have been grouped in books, edited by B l a k e m a n (1981) and W i n d e l s and L i n d o w (1985), and in EPPO Bulletin N° 4/1987 and N°1/1988.

Hyperparasites, mostly studied in different parts of the world, are various. In this, the decisive role belonged to distribution of primary parasite, climatic conditions, growing of certain cultivars, and cultivar susceptibility. So, for instance, in Yugoslavia *Cronartium ribicola* Fisch. represents, for now, a disease of insignificant danger, since *Pinus strobus* (and other species attacked by this parasite) are planted only as exotic plants; thus, their hyperparasite, *Tuberculina maxima* Rosti has not been recorded (J o s i f o v i ć, 1952). It is just opposite in the USA. There *Cronartium ribicola* causes great domages, so *Tuberculina maxima* is one of most frequently studies hyperparasites. *Polystigma rubrum* (Pers.) D. C. is very dangerous plum disease in Europe, especially in southeastern countries, where the cultivar „požegača" predominates. This is, probably, the reason that the most numerous papers on its hyperparasite - *Gloeosporium polystigmicolum* Bond. originate just from Russia (B o n d a r c e v, 1913; L u k y a n o v a,

1967; B o g o r i k, 1966), Bulgaria (T r i f o n o v a, 1934), Yugoslavia (J o s i f o v i ć, 1964; S t o j a n o v i ć & K o s t i ć, 1956). and others.

Similar is the situation with hyperparasite *T. persicina*. It has being find on a number of parasite fungi of fam. *Uredineales*, more less in the entire world. It was been treated according to the significance of primary parasites and effectiveness of hyperparasite. Since *Gymnosporangium fuscum* has recently been transferred to America, from that part of the world there are no more significant papers devoted to its hyperparasite. In Europe *T. persicina* is quoted as an example of hyperparasite on *G. fuscum*, but again, in this case the most significant papers originate from eastern Europe (G o b i,1885; V l a d i m i r s k a y a, 1939; M i t r o f a n o v a, 1970; 1971; H u l e a, 1939; M i j u š k o v i ć and V u č i n i ć, 1974).

The hyperparasite *T. persicina* in Montenegro occurs in large number; it is relatively widely distributed in convenient regions, sometimes it is 100% parasitic upon the primary pathogene. In sromata of *G. fuscum* the hyperparasite suppresses the primary parasite development, stopping their developmental cycle. *T. persicina* is strictly linked for parasite-host, therefore it cannot develop independently on plant organs.

In our studies, *T. persicina* did not sufficiently develop on any of examined artificial substances. Similar difficulty has also been experienced by H u b e r t (1934) in culturing *Tuberculina maxima* Rost. (author points out that *T. maxima* and *T. persicina* might be the same species). Similar to our experiments with *T. persicina*, B i r a g h i (1940) indicates that *Tuberculina sbrozzi* Car. et Sacc. as hyperparasite upon *Puccinia vincea* Berk. did not develop in artificial culture. Although the conidia easily germinate, growth of the inicial hypha stopped very rapidly. According to V a n i n (1938), one of the reasons for not applying the hyperparasites, is the difficulty to have them massively produced on artificial medium. K u h l m a n (1980) states that many hyperparasites grow slowly what in culture, what makes inoculum production for fild application difficult. Because of that, V l a d i m i r s k a y a (1939) has devoted a praticular attention to obtain *T. persicina* in pure culture and to choose the medium for its massive production. The author isolated *T. persicina* from *Puccinia graveolens* on *Cirsium arvense*. Spores of *T. persicina* from natural sustrate have been sown on malt-peptonic agar; in one of Petri dishes, abundant sporulation has been obtained, but further reproduction was unsuccessful. In a new series of experiments, several media have been examined. A

good sporulation was obtaind on carrot plugs, corn grains, grains of soya, beans, rice, on carrot agar milk, milk agar and agar of grape must. On other media the sporulation was weaker, and it was the weakest on the media containing 2% or more of pepton. The madia with greater content of sugar and lower contens of proteins have been more favourable for *T. persicina* massive production. Detailed presentation of V l a d i m i r s k a y a' s results here is given for two reasons: (a) some successful media of this autor did not give expected results in our investigations, and (b) in further researches both of some formerly used as well as some of the remaining ones should be examined.

Since V l a d i m i r s k a y a has not used *T. persicina* from *Gymnosporangium fuscum* but from an other parasite, our failure in culturing this hyperparasite might, eventually, be linked to the existence of physiological races of this hyperparasite. Hawever, M i t r o f a n o v a (1970) started with *T. persicina* sampled from *Gymnosporangium dobrozrakovae* Mitro, species which in some regions of former USSR, in addition to *G. fuscum*, is a parasite of pear. Cultivation was successful on the carrot plags and yeast agar.

Disregarding that we have not success in artificially culture of *T. persicina*, what was supposed to enable the investigation of application possibility of the hyperparasite in *G. fuscum* control, we have, starting directly from the spores of hyperparasite taken in natural conditions, proved its effectivness and established the best time for interventions. Of course, the warning of S p u r r (1985) that natural environment includes various interactions among nonpathogenic and pathogenic organisms and factors of the environment, should also be observed.

The authors dealing in more details with morphology and bioecology of *T. persicina*, give the results similar to the ones we have obtained; some of them have treated in more details ecological requiremets (V l a d i m i r s k a y a, 1939). M i t r o f a n o v a (1970) cites that, on Crimea, in zone where epiphytotia of pear rust occurs every year, natural infections by hyperparasite amount 40% in average, while in some years, like 1966, it was 100%.

CONCLUSIONS

A large number of antagonistic fungi were found in Montenegro. Most of them act as hyperparasites on primary pathogens of cultivated or spontaneously growing plants. One of the most common, most distributed and most effective hyperparasite proved to be *Tuberculina persicina* Ditm. on *Gymnosporangium fuscum* DC, very important pear parasite in southern part of Montenegro.

T. persicina may, in a very high rate, parasitize the aecial stromata of *G. fuscum*, sometimes 100%. The investigations carried out indicate that the infections can be achieved in the initial period of development of *G. fuscum* aecial stage, that is in stage of spermagonia, while on the stromata there still exist the characteristic exudate. Hyperparasite penetrates the stromata, stops and avoids the cycle of phytoparasite development. Instead of aecia, hyperparasite sporodochia are formed on stromata, evoluting subsequently into sclerotia. They either fall out of the leaf, or together with leaf fall to the ground and winter in that form.

Artificial inoculations by *T. persicina* spores gave very good results in *G. fuscum* control. Unfortunately, the attempts to culture this hyperparasite on artificial media have failed, and it will be necessary to have the investigations continued in this respect.

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TUBERCULINA PERSICINA (DESM.) SACC. , KAO HIPERPARAZIT NA GYMNOSPORANGIUM FUSCUM DC

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Sažetak

U posljednjih tridesetak godina sve se više posvećuje pažnja mogućnosti biološke borbe protiv prourzokovača biljnih bolesti, ali se još uvijek ne može govoriti o nekom odlučujućem napretku koji bi bitnije zamijenio klasične metode borbe protiv biljnih parazita, posebno na nadzemnim djelovima biljaka. Ipak, neke hiperparazitne gljive su se, u tom pogledu, pokazale vrlo perspektivnim.

Do skoro u Crnoj Gori nije bilo osmišljenijeg sistematskog rada na proučavanju antagonističkog odnosa gljiva kao faktora moguće biološke borbe, ali je konstatovan veći broj slučajeva njihove združene pojave. Od njih se, po značaju, izdvaja *Tuberculina persicina*, kao hiperparazit na *Gymnosporangium fuscum,* prouzrokovaču rđe na krušci.

G. fuscum je jedan od najznačajnijih parazita kruške u južnom dijelu Crne Gore. U Poljoprivrednom institutu u Podgorici, u periodu 1989-1992. paralelno su, u prirodnim uslovima, proučavani pojava i razvojni ciklus prouzrokovača rđe i njegovog hiperparazita, biološki i ekološki zahtjevi obje vrste, način i vrijeme diseminacije, način antagonističkog djelovanja i posljedice po primarnog parazita. Mogućnost uzgoja hiperparazita na vještačkim podlogama, vrijeme i način vještačkih inokulacija stepen eficijencije hiperparazita, smanjenje zaraznog potencijala u voćnjaku i, posebno, mogućnost praktičnog korišćenja hiperparazita u biološkoj borbi protiv fitopatogenog organizma - predstavljali su dalje ciljeve obavljenih istraživanja.

T. persicina može u vrlo visokom procentu, ponekad i 100%, parazitirati ecidijske strome *G. fuscum*. Infekcija se ostvaruje u početku razvoja ecidijskog stadijuma *G. fuscum*, u fazi spermagonija, dok na stromama

još postoji karakteristični eksudat. Hiperparazit prožima stromu, zaustavlja i prekida ciklus razvoja fitoparazita. Umjesto ecidija na stromama se stvaraju sporodohije hiperparazita, koje kasnije evoluiraju u sklerocije. One ili ispadaju iz lista kruške ili zajedno sa listom padaju na zemlju i u tom obliku prezimljuju.

Vještačkim inokulacijama sporama *T. persicina* dobijeni su vrlo dobri rezultati u suzbijanju *G. fuscum*. Na žalost, nijesu uspjeli pokušaji uzgoja ovog hiperparazita na vještačkim podlogama u mjeri koja bi obezbijedila masovno dobijanje spora, pa će biti potrebno da se proučavanja u tom pogledu nastave.

Fig. 1 - Pear leaf with severe natural *G. fuscum* infection

Fig. 2 - Orange pustules with *G. fuscum* pycnidia

Fig. 3 - Roestelia type of *G. fuscum* aecia

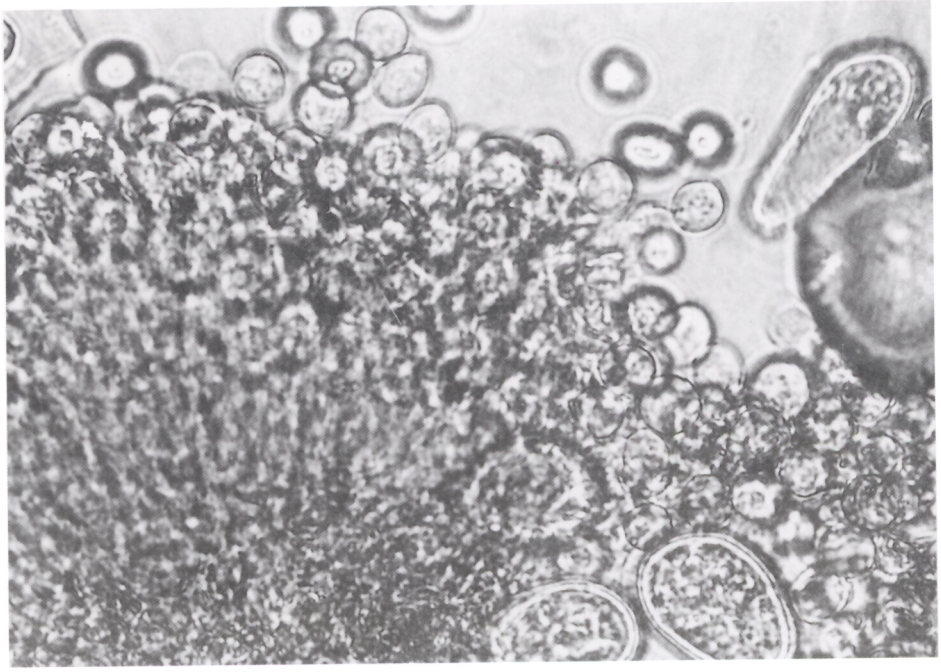

Fig. 4 - Sporodochia of *Tuberculina persicina*

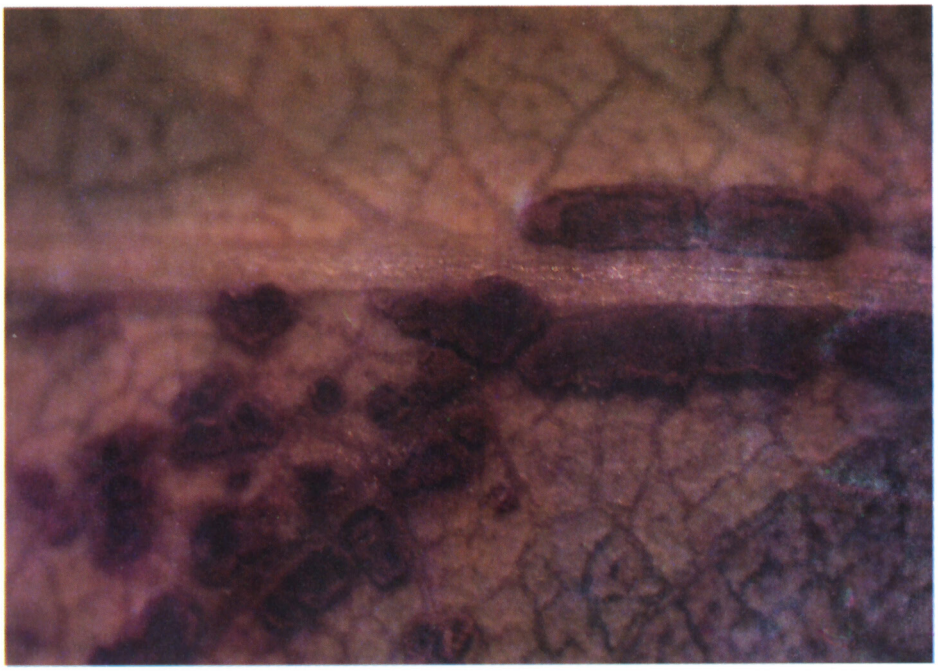

Fig. 5 - Sporodochia sprout by tearing pear leaf epidermis

Fig. 6 - *T. persicina* gradually removes and replaces *G. fuscum*

Fig. 7 - Sclerotia of *T. persicina*

Fig. 8 - Artificial inoculations of pear by *G. fuscum*