Minufiya J. Agric. Res. Vol. 2 No. 29: 401 - 414 (2004)

PARASITISM OF *Verticillium lecanii* ON PEA RUST (*Uromyces pisi*)

M. M. Saber

Plant Pathology. Department., Faculty. of Agriculture., Cairo University., Giza, Egypt

(Received: Mar., 9 , 2004)

ABSTRACT: *Urediospores of Uromyces pisi (Pres.) were obtained from naturally infected leaves of pea (Pisum sativum L.) plants, cv. Little Marvel, which showed typical symptoms of pea rust disease. Verticillium lecanii (Zimm.) Viegas, was found infesting uredinia of Uromyces pisi, the causal organism of pea rust. V. lecanii was a destructive mycoparasite of the pea rust. Reduction of the level of pea rust on potted plants kept in the glasshouse was demonstrated when V. lecanii was present. It was also found that when V. lecanii and U. pisi were applied to pea leaf as mixed inoculum the level of rust was lower than when the rust fungus was applied alone.*

The interaction between Verticillum lecanii and Uromyces pisi growing on pea leaves, was studied. Light and electron microscopy were used to provide evidence for the parasitism of V. lecanii on rust spores.

On agar cultures, germ tubes of V. lecanii did not attack germ tubes of U. pisi , however hyphae of V. lecanii grew within and around urediospores in sori. Within seven to ten days after inoculation, the hyperparasite did not penetrate the sporogenous tissue of the rust fungus or into the leaf tissue. Electron microscopy showed that V. lecanii preferably attacked the urediospore walls and spore contents of U. pisi. The pellicle and the spines of the urediospores remained intact. Light intensity had a positive effect on the development of V. lecanii in the pustule of U. pisi. Good develop- ment of the hyperparasite occurred between 16°C-20°C, and 90% - 100% air humidity.

Key words: *Pisum sativum L., Uromyces pisi , Verticillium lecanii, Interaction, Biological control*

INTRODUCTION

Pea rust disease incited by *Uromyces pisi* (Pers.), occurs annually through out the pea growing areas of Egypt. It was considered as one of the major diseases of pea and it was a potential threat to pea production in Egypt. (Khafagi *et al.*, 1995 and Abada *et al.*, 1997). Recently, increasing attention has focused on biological control of insect pests and plant diseases (Askary *et al.*, 1997; Minaas 2001 and Aly *et al .*, 2002). A number of different fungal hyperparasites had been observed to grow in pustules of

rust fungi. Examples of these were *Verticillium* sp. (Spencer 1980), *Darluca filum* (Kranz 1973) *Monocillium nordinii* (Tsuneda and Hiratsuka 1980), *Alternaria* and *Cladosporium* species (Omar and Heather 1979).

Verticillium lecanii (Zimm.) Viegas had a wide host range; it parasitizes scale insects and aphids and it was reported to colonize cultures of *Erysiphe graminis* DC., the causal organism of barley mildew, and of *Uromyces appendiculatus* (Pers.) Ung., the cause of bean rust. (Spencer 1980).

Mckenzie and Hudson (1976) found *V. lecanii* to be a common mycoparasite of wheat stem rust caused by *Puccinia graminis* Pers. f.sp. *tritici*, but also attacked different insects (Hall 1980). *V. lecanii* used as a biological control agent of carnation rust, *Uromyces dianthi* (Spencer 1980). Few studies on the mode of action of the parasitism of *V. lecanii* were done. Lysis of urediospore germ tubes (Garcia Acha *et al.*, 1965), bursting of urediospores of coffee rust, *Hemileia vastatrix* (Silveira and Rodrigufs 1971) and penetration into urediospores of stripe rust, *Puccinia striiformis* (Schroeder and Hassebrauk 1957) by *V. lecanii* had been described. After infection of pustules of bean rust (*Uromyces phaseoli)*, the hyperparasite remained restricted to the pustule area and did not penetrate into the bean leaf tissue (Mendgen and Casper 1980).

The aim of the present work is to study occurrence of *V. lecanii* on *U. pisi* and the effect of *V. lecanii* on the level of pea rust and also studies on the interactions between *U. pisi* and *V. lecanii* and the influence of temperature, light and air humidity on the development of the hyperparasite in the rust pustule.

MATERIALS AND METHODS

1. Fungi and plant material

1.1. Samples

Pea (*Pisum sativum* L.) plants naturally displaying symptoms of pea rust disease were originally obtained from different fields of El-Giza governorate during the growing season 2000 to provide the inoculum (Fig. 1A).

Pea seedlings (7 days old) of highly susceptible cultivar (Little Marvel) were grown in 10 cm diameter sterilized plastic pots (10 plants/pot). The seedlings of pea were dusted by freshly urediospores of *Uromyces pisi* which mixed with talcum powder (1:20 v/v).These plants were raised at $16\pm1^{\circ}C$ and illuminated with fluorescent light, 40900 lux, 18 h/days unless otherwise indicated.

Air humidity was controlled by pumping the air that surrounded individual plans through gas washing bottles containing saturated salt solution (Winston and Bates 1960).

1.2. Inoculum of fugni

a- Inoculum of *U. pisi*

The host rust fungus, *U. pisi* were obtained from infected pea plants in the above mentioned greenhouse.

b- Inoculum of *V. lecanii*

The isolate of *V. lecanii* was found colonizing uredinia of pea rust. It was isolated from a pea rust pustule (Fig. 1B). The organism was maintained at 20°C on Czapek-Dox agar. Its identity (mean spore length 3.2 μm, mean spore diameter 1.8 μm). Ten-day-old cultures were used to provide conidial suspensions (5×10^5 ml^{-1} water) to study its effect on *U. pisi* on pea plants.

2. Biological studies

This biological experiments was carried out to determine the reduction of rust disease in the presence of *V. lecanii.* In this case, 150 pea plants c.v little Marvel, were used to carry out this biological assay. The pea plants were divided into three groups each group consisted of 50 plants which potted in separate cages (1.0m x 1.0m x 1.5m) in the glasshouse. Seven days from sowing in sterilized plastic pots 10 cm in diameter (5 plants/pot), the seedlings of different groups were sprayed, with spore suspension of the different fungi as follows:

First group (gp.I) inoculated with urediospores ($c.10^5$ mL^{-1}) of *U. pisi* alone. Second group (gp.II) inoculated with mixed urediospores ($c.10^5 mL^{-1}$) of *U. pisi* and conidia ($5 \times 10^5 mL^{-1}$) of *V. lecani.* Third group (gp.III) kept as control without any fungal inoculation to judge any external infection. In each case 0.05% Teepol was added. In all treatments plants were sprayed late in the afternoon, floor of the house was thoroughly wetted and the door and vents were left closed overnight in order to maintain relatively high humidity. Daily observations were carried out to record appearance and counting the pustules for 14 days from inoculation (Spencer 1980)

3. Serology

The antiserum against *V. lecanii* was prepared as earlier described (Casper and Mendgen 1979). For the enzyme linked immunosorbent assay (ELISA), the method of Clark and Adams (1977) was adopted. To measure the amount of *Verticillium* hyphae in rust infected plant tissues, 200 mg of the infected leaves were freeze-dried and subjected to the ELISA test. The absorbance values (A_{405}) were used to indicate the amount of *V. lecanii* in the leaf tissue. For immunofluorescence, samples of the infected leaves were fixed in 2% glutaraldehyde and embedded in paraffin. After sectioning and removal of the paraffin, sections were washed in 0.01 M phosphate buffer, pH 7.2 with 0.15 M NaCl. Incubation of sections was performed in antiserum, diluted 1:50 with buffer. After thorough washing in buffer, the reaction with the antiserum was made visible by incubation in fluorescein-conjugated anti-

rabbit-Ig from sheep (Miles) diluted with buffer 1:20. Photographs were taken with a Leitz fluorescence microscope using filter blocks A, D, I2 and Ilford FP4 film (Mendgen and Casper 1980).

4. Light- and electronmicroscopy

Low-power light microscopy was carried out on fresh, unstained eruptive uredinia of the rusts, either healthy or infected with *V. lecanii.* To study the interaction of germ tubes of both fungi, 2% water agar was spread on a slide microscope and the spores were sprayed on the thin agar layer. The agar was covered with a glass cover. To keep a distance between the glass cover and agar surface, bits of glass were put on the agar so that a thin layer of air remained around the spores and their germ tubes.

For electron microscopy of pustules, leaves were sprayed with a spore suspension of *V. lecanii* when the pustules of pea rust appeared. The plants were then kept under a plastic cover to keep high humidity. Ten days later, when a white weft of sporulating *Verticillium* mycelium could be seen over the uredia, samples were taken for electron microscopic studies. These samples were fixed in2% glutaraldehyde and in 2% osmium tetroxide, dehydrated in alcohol, and embedded in Spurr's epoxy resin. Sections were made with glass knives, stained with uranylacetate and lead citrate and examined with a Zeiss EM 10 CR electron microscope. Micrographs were taken after examination using Electron microscope in EM unit, specialized Hospital Ain Shams University.

5. Statistical analysis

Averages were compared level of probability using least significant difference L.S.D. (Fisher 1948).

RESULTS

Isolation trials carried out from leaves formed on Pea (*Pisum sativum* L.) plants cv. Little Marvel showing typical symptoms of pea rust disease (Fig. 1A) and *V. lecanii* which was isolated from a pea rust pustule (Fig. 1B).

1. Biological studies

The level of rust infection on plants in the glasshouse was assessed 2 weeks after inoculation as the total number of eruptive uredinia on each plant (Table 1). There were marginal differences in the mean numbers of uredinia which developed on each plant. This ascribed to differences in plant size or to differences in environmental conditions during the infection and inocubation periods, but in all cases, when *V. lecanii* was present the level of pea rust disease was reduced to 10-15% of the level in its absence. At this time *V. lecanii* could be seen growing on the erupment uredinia (Fig. 1c).

Lower power light microscopy or eruptive rust uredinia infected with *V. lecanii* showed the rust spores heavily overlaid with dense wefts of *V. lecanii* mycelium (Fig. 1c).

Table (1): Average number of uredinia per plant on pea plants sprayed with sporesuspension of different fungi.

Group Number	Treatments*	Number of eruptive uredinia**
Group (I)	*U. pisi* alone	14.4
Group (II)	*U. pisi* + *V. lecanii*	2.3 #
Group (III)	Non-inoculated (control)	0

N.B. the percentage of the pustuls reduction was 16.0%.
* Treatment (inoculated plants with the different fungi).
** Mean of 50 plants.
# Significantly lower than *U. pisi* alone at P – 0.01.

2. The interaction between germ tubes of *Uromyces pisi* and *Verticillium lecanii.*

To determine whether *V. lecanii* was able to attack the rust fungus during its growth on the leaf epidermis, spores of *V. lecanii* and *U. pisi* were sprayed on a thin agar layer and observed under the microscope for periods of several days at 16°C and 100% humidity.

The pea rust urediospores germinated earlier and the germ tube grew much faster as compared to *V. lecanii.* Therefore, spores of *V. lecanii* were sprayed on agar 24 h in advance of those of *U. pisi* and were observed during the following 7-10 days. Neither urediospores nor germ tubes of *U. pisi* attracted the germ tubes of *V. lecanii.* The hyperparasite usually passed the germ tubes of the rust fungus (Fig. 1D) and, in case it met a urediospore, tended to branch and to grow on the surface of the urediospores of *U. pisi* (Fig. 1E). Urediospores became disorganized 3 to 4 days after they were in contact with the hyperparasite (Fig.1F). About 7 days after the contact of *V. lecanii* and the urediospores, condiospores, of *V. lecanii* grew out of the urediospores (Fig. 1G).

3. The interaction of *Verticillium lecanii* and *Uromyces pisi* within host leaves.

Uredia of pea rust infected with *V. lecanii* generally were covered with a white web of the hyperparasite mycelium four to seven days after inoculation with *V. lecanii.* Ten days after inoculation, cross sections through the pustule area showed large numbers of *Verticillium* hyphae present between the urediospores of the rust fungus. With light microscopy, it was not possible to differentiate between the hyphae of the rust fungus and those of

the hyperparasite within the leaf tissue. Therefore, the hyphae of *V. lecanii* were labeled with fluorescing antibodies. At this treatment of the paraffin sections, hyphae of *V. lacanii* gave a bright fluorescence and they were observed on the epidermal surface around the pustule and in the pustule, between and within the urediospores. They were not detected within the leaf tissue nor in the sporogenous tissue of the rust fungus. (Fig. 1H) shows such a cross section after blue light excitation (340-380 nm), which demonstrates the hyphae and urediospores of the pea rust fungus. The same section, after selective excitation for fluorescein conjugated antibodies, demonstrates the hyphae of the hyperparasite, which were restricted to the urediospore layer (Fig. 1I). Cross section examined with the electron microscope confirmed this observation.*Verticillium* hyphae were, with few exceptions, restricted to the urediospore layer of the uredium. The spore walls of the urediospores became dissolved, following contact with *V. lecanii* hyphae. The dissolution of the wall layer was restricted to the spore wall (Fig. 1J).

The pellicle and the spines of the spore were not degraded. The process of wall degradation proceeded centripetally. Some areas in the urediospore layer of the pustule showed spores which were surrounded and penetrated by a large number of *V. lecanii* hyphae (Fig. 1K). In these urediospores, the spore wall was completely degraded, but the pellicle and the spines remained intact. The content of the urediospores seemed to be only gradually degraded.

4. Growth conditions for *Verticillium lecanii* in the rust pustule

Since *V. lecanii* is specialized to grow within a rust pustule, it seemed important to define the optimal conditions for the hyperparasite to grow in the uredium. To measure the amount of *V. lecanii* hyphae within the pustule, a serological method was used as proposed by (Casper and Mendgen 1979), with the enzyme linked immunosorbent assay (ELISA), only *V. lecanli* hyphae reacted. The amount of hyphae of the hyperparasite was measured after variation of air humidity, temperature and light intensity. In all experiments, plants were inoculated with *V. lecanii* when sporulation of *U. pisi* had begun and samples of the infected pea leaves were taken even days later. For every variant 200 mg of the freeze-dried infected leaves were subjected to the ELISA-test. From this test, it was obvious that the growth of the hyperparasite was mainly dependent on air humidity. Greater than 80% relative humidity was required for growth of *V. lecanii*. Relative humidity of 95 to 100% was optimal (Fig. 2). The influence of temperature was not very specific. A range of temperatures between 16°C and 20°C allowed good growth (Fig.2). Higher temperatures were not tested as they did not allow good development of pea rust under the given conditions. Light had a positive effect on the development of *V. lecanii* in the rust pustule (Fig. 3).

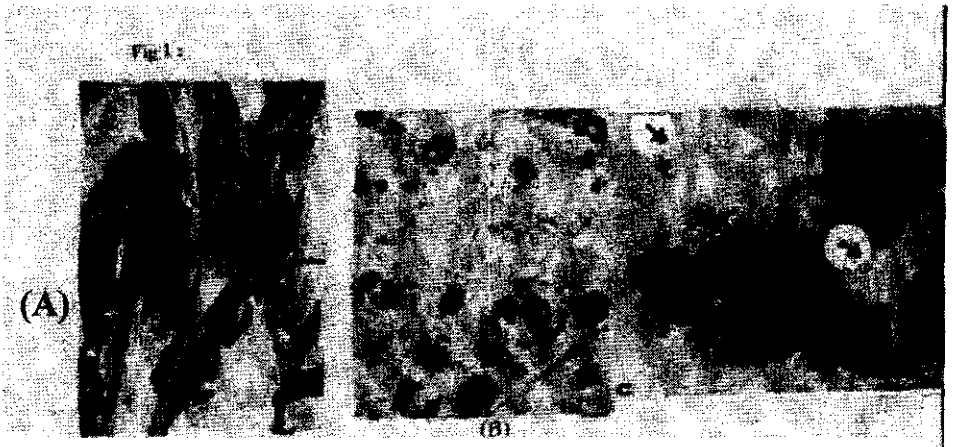

Fig. (1): Naturally infected pea plants showing (A) symptoms of rust caused by *U. pisi* (B) leave of pea with *V.lecanii* growing on coalesced uredinia of *U.pisi*.(C) Low- power light micrograph of erupmen uredinia of *U.pisi* overgrown with *V. lecanii*

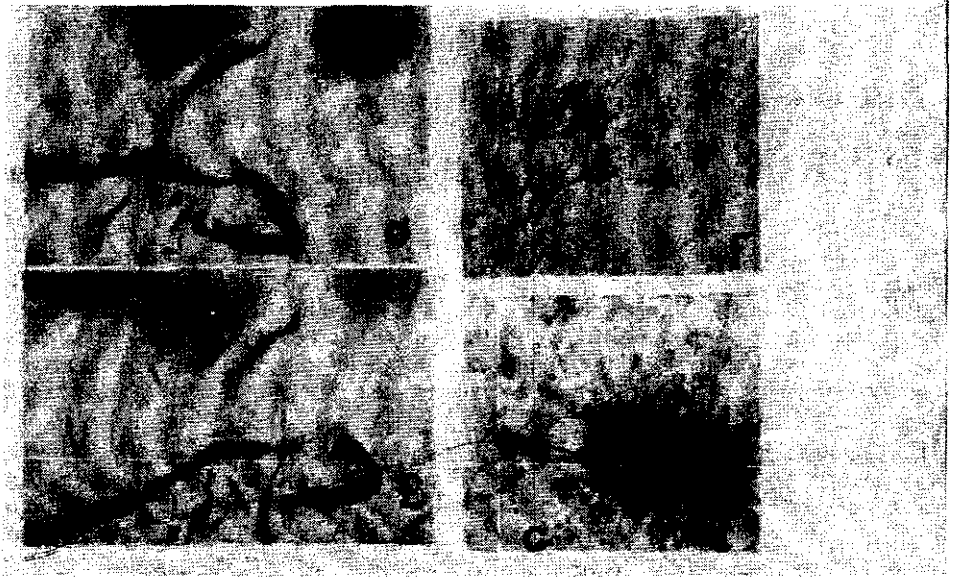

Fig: (D): *Verticillium lecanii* spores with their germ tubers, 48h old, on water agar. The germ tube of *Urimyces pisi* is growing between the hyphae of the hyperparasite, is 24h old. Urediospores (U) of *U. pisi* (x 400). Fig. (E): The same preparation as Figure D, but 24h later. The germ tube of *U. pisi* terminated growth. The germ tubes of *V. lecanii* grew past the rust germ tube on to the urediospore (arrow) (x 400). Fig. G. Fig. F: A similar preparation as in Fig. D: four days later. The urediospores have collapsed (x 150). Fig. G: Conidiophores of *V. lecanii* grew out of urediospores about 7 days after the contact between *V. lecanii* and *U. pisi* (x 150)

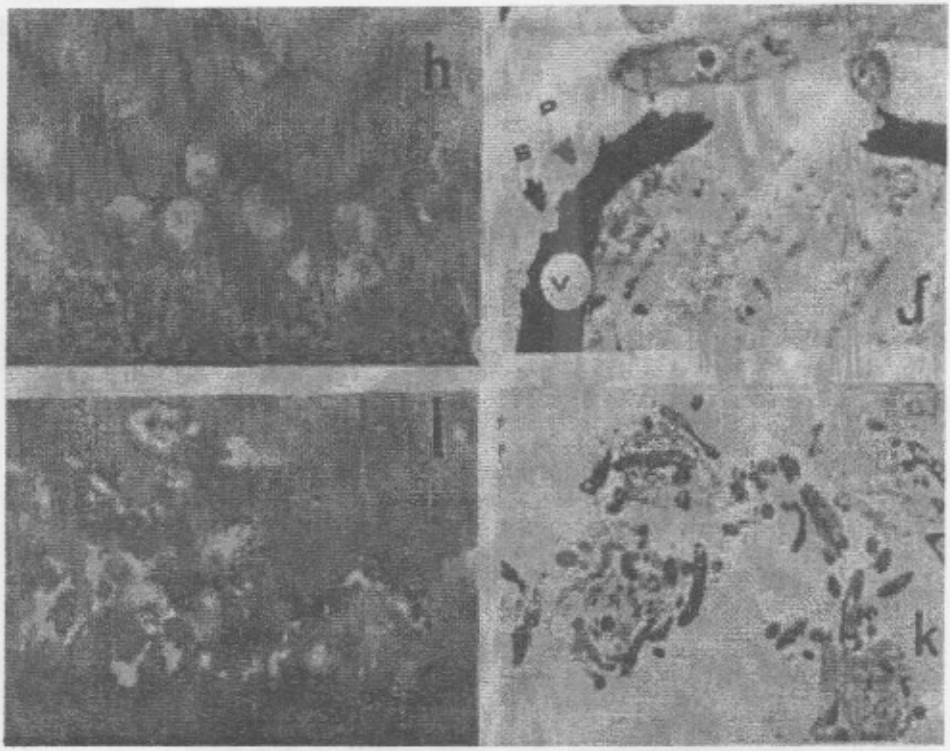

fig. (H): Cross section through a pea rust (*U. pisi*) pustule, ten days after inoculation with *V. lecanii*. The section was treated with fluorescein conjugated *V. lecanii* antibodies. After blue light excitation (340-380 nm) only hyphae and urediospores of the pea rust fungus can be seen (x 530). Fig. (I): The same section as in Fig. H, except that the fluorescein-conjugated antibodies against *V. lecanii* can be seen after selective excitation (430-470 nm) for fluorescein indicating the hyphae of the phyperarasite (x 530).

Fig. (J): Partial degradation of a urediospore wall (V) in contact with *V. lecanii* hyphae. The pellicle (P) and the spines (S) remain intact. The sample was taken from a pea rust pustule ten days after inoculation with *V. lecanii* (x 6000). Fig. (K): Urediospores of *U. pisi* and hyphae of *V. lecanii*. A similar sample as in Fig. G., but the urediospore wall is completely degraded. Only a part of the pellicle, the spines and the spore content are still visible. The hyphae of the hyperparasite have penetrated the urediospores at numerous places (x 2700).

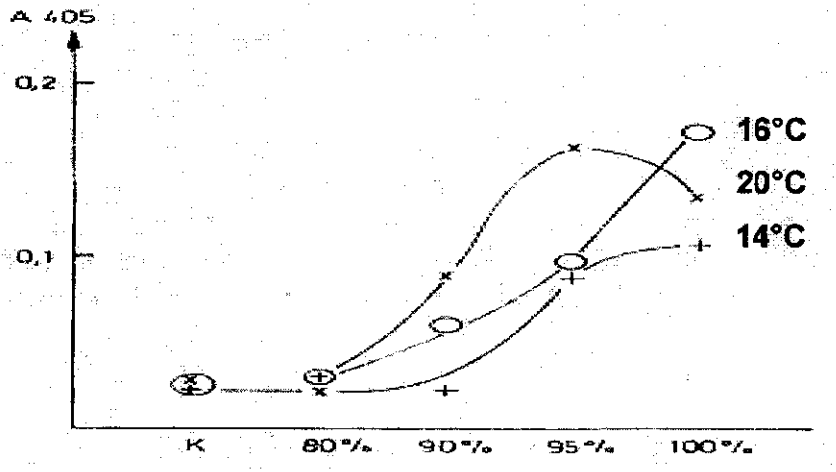

Fig. (2): Influence of temperature and air humidity on the development of *V. lecanii* grown under 4000 lux

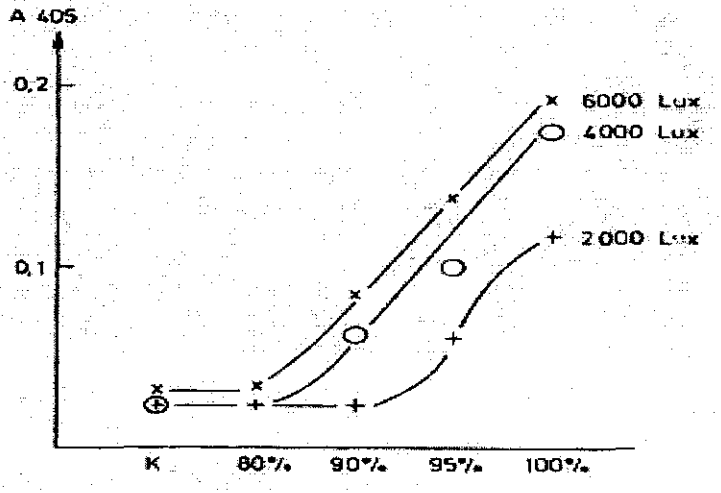

Fig. (3): Influence of light and air humidity on the development of *V. lecanii* grown at 16°C.

DISCUSSION

V. lecanii is specialized to parasitize the urediospores of *U. pisi*. This observation confirms results in similar host-parasite-hyperparasite system (Schroeder and Hassebrauk 1957; Garcia Acha 1965; Silveira and Rodriguez 1971). The reason for this specialization may be related to its ability to degrade the spore walls of rust urediospores.

The mechanism of wall degradation is still unknown. It is also unknown, why the spore content is broken down to a lesser extent than the urediospore walls. It may be speculated that *V. lecanii* possesses a very efficient chitinase or other wall degrading enzymes. This speculation is supported by the fact that *V. lecanii* attacks both spore walls and insects. Both have chitin as the major structural constituent (Hall 1980).

This study gives some indication on the value of *V. lecanii* for the biological control of rust fungi. Spencer (1980) showed that on rust-inoculated carnation plants, placed in a humidity chamber to encourage infection, rust was reduced by 50% in the presence of *V. lecanii*. It has now been shown that under glasshouse conditions, pea rust was significantly reduced when *V. lecanii* conidia were present in the suspension of urediospores of *U. pisi* used as inoculum (Table 1). The hyperparasite is restricted to the area of the rust uredium and does not penetrate into the leaf of the host plant. This corresponds to observations in bean rust (*Uromyces phaseoli*) infected leaves (Mendgen and Casper 1980). Increasing light intensity and temperature favor the growth of the hyperparasite, these conditions may also favor the plants development and consequently the rust fungus development, these results are of minor importance if the hyperparasites value for biological control is appreciated. More important is *Verticillium's* requirement of more than 80% air humidity for growth. This restricts the value of *V. lecanii* as a bio-control agent to geographical areas with high humidity. The results may explain different findings with *V. lecanii* as a control agent. The glasshouse experiments indicate that the use of *V. lecanii* can provide a measure of control of *U. pisi*. The level of this control might be improved if it could be ensured that *U. pisi* was more effectively exposed to attack by *V. lecanii* by the use of wetting agents to aid dispersion of the *V. lecanii* conidia. The widespread natural occurrence of *V. lecanii* on the nursery also indicates that careful manipulation of the glasshouse environment might be used to achieve better control of rust. If *V. lecanii* sprayed on to the crop as a means of biological control the indications are that it would be used as a prophylactic rather than as a protectant since the conidia applied to plants more than 24 h before inoculation with rust gave no benefit. It would seem therefore that conidia are either inherently short-lived or are sensitive to environmental stresses such as desiccation.

The protection of the environment is receiving ever-increasing attention and the possibility of controlling plant disease by biological means rather than with chemicals thus merits serious consideration. The protected environment of the glasshouse lends itself particularly well to the use of biological control agents, and further work on the natural of the inhibition of *U. pisi* by *V. lecanii* is justified, as is a study of the level of rust control to be achieved in commercial growing conditions. If *V. lecanii* were to be shown to be particularly attractive in biological control, the fact that it is of widespread occurrence in commercial pea houses should have some bearing on deliberations concerning its clearance under existing safety regulations.

Spencer (1980) reported successful control in greenhouse experiments. Obviously, it is important that environmental conditions are rigidly controlled for successful control of a rust fungus with *V. lecanii*.

REFERENCES

Abada K.A.; M.M. Saber and M.A. Moustafa (1997). Control of pea rust disease under Egyptian conditions. 8th Congress of the Egyptian Phytopath. Soc., Cairo 199-210.

Aly.A. Z.; H. Buchenauer; M.I. Abou.Zaid; M.S. Shalaby and M.M. Atia (2002). Induced resistance against tomato late blight disease using bioagents . Egypt. J. Phytopathol., Vol. 30, No. PP. 25-43.

Askary H.; N. Benhamou and J. Brodeur (1997). Ultrastructural and cytochemical investigations of the antagonistic effect of V. lecanii on cucumber powdery midew. Amer . Phyto Society . 87(7) 359-368.

Casper R. and K. Mendgen (1979). Quantitative serological estimation of a hyperparasite: Detection of Verticillium lecanii in yellow rust infected wheat leaves by ELISA. Phytopath. Z. 94: 89-91.

Clark M.F. and A.N. Adams (1977). Characteristics of the microplate method of the enzyme-linked immunosorbent assay (ELISA) for the detection of plant viruses. J. Gen. Virol. 34: 475-483.

Fisher R.A. (1948). Statistical Methods for Research Workers. Oliver and Boyd, London.

Garcia Acha I.; J.A. Leal and J.R. Villanueva (1965). Lysis of uredospore germ tubes of rusts by species of Verticillium. Phytopathology 55: 40-42.

Hall R.A. (1980). Laboratory infection of insects by Verticillium lecanii strains isolated from phytopathogenic fungi. Trans. Brit. Mycol. Soc. 74: 445-446.

Khafagi Y.S.; M.M. Abo El-Ella; S. Ahmed Fatma and M. El-Gizy Samira (1995). Pea rust in Egypt. Menofiya J. Agric.Res., 20: 2155-2170.

Kranz J. (1973). A host list of the rust parasite Eudarluca caricis (Fr.) O. Eriks. Nova Hedwigia . 24: 169-180.

Mckenzie E.H.C. and H.J. Hudson (1976). Mycoflora of rust infected and non infected plant material during decay. Trans. Brit. Mycol. Soc. 66: 223-238.

Mendgen K. and R. Casper (1980). Detection of Verticillium lecanii in pustules of bean rust (Uromyces phaseoli) by immunofluorescence. Phytopath. Z. 99: 362-264.

Minaas, E.A. Sallam (2001). Scanning micrography in evaluation of leaf rust biological control. Egypt, J. Phytopathology., Vol. 29 No. 1. pp. 11-20.

Omar M. and W.A. Heather (1979). Effect of saprophytic phylloplane fungi on germination and development of Melampsora larici-populina. Trans. Brit. Mycol. Soc. 72: 225-231.

Silveira H.L. and C.R. Rodriguez Jr (1971). Bursting of rust uredospores caused by Verticillium hemileiae Bour. culture filtrates. Agron. Lusit. 33: 391-395.

Spencer D.M. (1980). Parasitism of carnation rust (Uromyces dianthi) by Verticillium lecanii. Trans.Brith. Mycol. Soc. 74: 191-194.

Schroeder H. and K. Hassebrauk (1957). Beitrage zur Biologie von Darluca filum (Bio.) und einigen anderen, auf Uredinen beobachteten Pilzen. Zbl. Bakt., II. Abt., 110: 676-696.

Tsuneda A. and Y. Hiratsuka (1980). Parasitization of pine stem rust fungi by Monocillium nordinii. Phytopathology 70: 1101-1103.

Winston P.W. and Bates D.H. (1960). Saturated solutions for the control of humidity in biological research. Ecology 41: 232-237

تطفل الفطر *Verticillium lecanii* على الفطر *Uromyces pisi* المسبب لمرض الصدأ فى البسلة

مجدي محمد صابر

قسم أمراض النبات – كلية الزراعة –جامعة القاهرة

الملخص العربى

تم الحصول على الجراثيم اليوريدية للفطر ***Uromyces pisi*** من على أوراق نباتات البسلة صنف ليتل مارفل والتي كانت مصابه إصابة طبيعية حيث اظهرت أعراض نموذجية لمرض الصدأ. وقد لوحظ وجود فطر آخر مشارك مع البثرات اليوريدية لمرض الصدأ ، وقد تم عزل هذا الفطر المصاحب لهذه البثرات وذلك بعد تنميته على بيئة تشابكس أجار ديكستروز ، باستخدام الفحص الميكروسكوبي ، تم التعرف على أنه الفطر ***Verticillium lecanii*** ذو الجراثيم الكونيدية بمقياس (٣,٢ طول × ١,٨ عرض) ميكرون. تم دراسة التفاعل بين هذا الفطر ***V. lecanii*** والفطر ***U.pisi*** المسبب للصدأ في البسلة حيث اثبتت الدراسة أن الفطر ***V. lecanii*** ذو قدرة تطفل عالية على الفطر ***U. pisi*** حيث اتضح من التجارب أن هناك انخفاض في مستوى شدة الاصابة بمرض الصدأ على نباتات البسلة التي زرعت في الصوبة الزجاجية وذلك في مجموعة النباتات المحقونة بالفطر ***V. lecanii*** مع الفطر ***U. pisi*** معاً مقارنة بمجموعة النباتات المحقونة بفطر الصدأ ***U. pisi*** بمفرده. وقد أظهرت نتائج الفحص الميكروسكوبي الضوئي والالكتروني واختبارات الاليزا أن الفطر ***V. lecanii*** يتطفل على الفطر ***U. pisi*** المسبب للصدأ فقد وجد على مزارع الاجار أن أنبوبة الانبات للفطر ***V. lecanii*** لم تهاجم أنبوبة انبات الفطر ***U. pisi*** بينما هيفات الفطر ***V . lecanii*** نمت داخل وحول الجراثيم اليوريدية في البثرة. وقد أظهرت أيضا نتائج الفحص بالميكروسكوب الالكتروني أن الفطر ***V. lecanii*** يهاجم جدر ومحتوى الجراثيم اليوريدية للفطر***U. pisi*** ومن التجارب التي اجريت لمعرفة العوامل المؤثرة على قدرة وتطور التطفل وجد أن هناك تطفل جيد حدث وذلك عند كثافة أو شدة الضوء ودرجات حرارة ما بين ١٦–٢٠ درجةمئويه) وما بين ٩٠–١٠٠% رطوبة.

وتشـــير نتائج هذه الدراسة إلى أن استخدام الفطر ***Verticillium lecanii*** ممثلاً للمكافحة الحيوية لمرض صدأ البسلة يعتبر ذلك من التقنيات الحديثة الآمنة ذات الكفاءة العالية والتـــي يمكن تطبيقها فيما بعد تحت ظروف الحقل لمكافحة مسببات الأمراض بالاضافة إلى أنها بديـــلاً لاســـتخدام المبيدات التي تدخل ضمن تلوث البيئة والتي تكون ذات أثر سيئ على صحة الإنسان وكفاءته. والتى تقلل من التكلفة الاقتصادية لانتاج البسلة في مصر.