HISTOPATHOLOGY OF POWDERY MILDEWED TOMATO LEAVES AS AFFECTED BY HOST SUSCEPTIBILITY

AWAD N.G.H., M.F.I. TADROUS AND M.S. ABD EL-MEGID

Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt

(Manuscript received 7 October 2003)

Abstract

The causal pathogen of tomato powdery mildew disease was identified as *Leveillula taurica* (Lév.) Arnaud according to the symptoms and the morphological characteristics of the fungal conidiophores and conidia .

Conidia often germinate at the basal or lateral sites forming uni- or multi germ tubes. Conidial germination was better on tomato leaf strips under moist atmosphere than in free water or dry conditions.

Number of conidiophores and their branching depend on the susceptibility of tomato cultivar. Few uni and unbranched conidiophores in the least susceptible cv. Super strain-B and multi and occasionally branched conidiophores in the highly susceptible cv. Strain-B were observed. However, intermediate number of conidiophores and conidia formed in the lower surface of the moderately susceptible cv. Castle Rock.

Examination of conidial germination revealed that spore germ tubes are initiated after 6 hr. from inoculation under warm and wet conditions and their elongation extends from 12-24 hr. towards the stomata of the upper leaf surface. Well developed symptoms appeared 7 days after inoculation under greenhouse conditions at 26°C±2 on tomato plant leaves simultaneously with that inoculated detached leaves *in vitro*.

INTRODUCTION

Powdery mildew is an important disease which seriously attacks tomato plants in both the open fields and under low tunnels during foliage growth particularly after flowering through late summer and/or autumn (Nili) tomato plantations all over Egypts

The disease often causes considerable reduction in tomato foliage and marked reduction in the quality and quantity of harvested tomato (Cerkauskas *et al*, 2000 and Haroun, 2002).

Disease importance markedly increased with the expansion in growing tomato hybrids tolerant to white fly (*Bemisia tabaci*) transmitting the yellow tomato leaf curl virus (YTLCV) during the Nili plantations in Fayoum province. Ty group hybrids (Ty-20; Ty-70; Ty-84 and Ty-317) were the most susceptible to powdery mildew in Fayoum, Ismailia and Sharkia provinces.

Leveillula taurica is capable of infecting all commercial tomato cultivars and the new hybrids to different degrees. Haroun (2002) classified the conventional and processing tomato cvs. cultivated in Egypt into three categories i.e. highly susceptible (Supermarmande, Strain-B, Marconi and Super green); moderately susceptible (Peto-86 and Castle Rock) and least susceptible (UC-97/3, Cal Ace, Ace and Super strain-B) cvs. The reaction of many tomato cvs. against the disease was previously investigated (Georgiev *et al* , 1993; Karaseviez and Zitter , 1996; Ciccarese *et al*, 1998 and Huang Caicheng *et al* , 1998).

The objective of this research was to study the conidial germination of the pathogen *in vitro* and *in vivo* (on leaf strips and plant leaf tissues). Also, the reaction of three tomato cvs. differing in their susceptibility to the powdery mildew disease infection (Haroun , 2002) was histopathologically studied.

MATERIALS AND METHODS

Sample collection and identification of the pathogen

Leaf samples of tomato Ty-20 hybrid cultivated in Fayoum province (Tammya locality) during autumn (Nili) plantations (2002) were collected. The infected leaves were examined to describe the symptoms and morphological characteristics of the causal organism. Minimum, maximum and average dimensions (in µm) of 50 conidia and conidiophores and mycelium diameter were determined to identify the causal pathogen according to El-Fiki and Fawzy (1987) KangSooWoong *et. al.* (1995) and Cerkauskas *et .al* . (1999).

Isolation, purification and pathogenicity

Conidia on the lower surface of seventy day-old tomato leaves (Ty-20 hybrid), showing powdery mildew disease symptoms, were dusted on the upper surface of

healthy Ty-20 tomato leaves (at the age of 60 days), covered with plastic cage and the plant canopy were kept wet under greenhouse conditions at 26°C±2 for two days (Sadasivan and Ellingboe, 1962). Incubation was made under the same greenhouse conditions until typical powdery mildew symptoms appeared.

Conidial germination *in vitro*

Four different spore germination techniques were employed by fixing a ring on glass slide using Canada balsam (A and B) and sterilized with ethyl alcohol. The slide was placed on V-shape glass rod in a sterilized Petri dish containing wet filter paper (10 ml water) (C and D) . The slides were prepared as follows and shown in Figure (1):

A) Conidia were dispersed on a piece of cellophane sheet 3 x 5 cm which covers a ring containing water.

B) Conidia were dusted on water inside the ring .

C) Conidia were dusted on the upper epidermal strips of tomato leaf.

D) Conidia were dusted on a dry glass slide .

Average percentage of conidial germination was calculated after 24 hr at 25°C.

Stages of conidial germination *in vivo*

Sixty-day old Ty-20 hybrid tomato leaves were marked with a marker to delineate the sites of inoculation then inoculated with *L. taurica* conidia as mentioned before. The test plants were kept under greenhouse conditions at 26°C±2. Leaf epidermal strips of inoculated sites (2 cm^2) were prepared (Schnathorst, 1959) at 6, 12, 18, 24 hr. and 7 days after inoculation. Each strip was placed in a drop of water on sterilized glass slide and microscopically examined for conidial germination and tissue penetration.

Histopathological reaction of tomato cultivars differing in their susceptibility to powdery mildew

Transverse sections in healthy and powdery mildewed tomato leaves of highly susceptible (Strain-B); moderately susceptible (Castle rock) and least susceptible (Super strain-B) were made 15 days following inoculation under greenhouse conditions at 26°C±2. Samples of tomato leaves (1 cm^2) of each cv. were killed and fixed in formalin acetic acid-alcohol solution (F A A), dehydrated, clarified with ethyl-

alcohol-xylene and embedded in pure paraffin wax (M.P. 56-58°C). Then, samples were transversally sectioned (15 microns thickness) using a rotary microtome and double stained by safranin-light green according to Johansen (1958). Sections were microscopically examined for pathogen behaviour and changes in the mesophyl tissue due to infection.

RESULTS AND DISCUSSION

Typical tomato natural powdery mildew disease symptoms were observed as light green to bright yellow lesions on the upper leaf surface as well as powdery fungal sporulation on the lower surface of the leaf which often coalesce in the advanced stage. Finally, golden yellow lesions on the upper surface and necrotic lesions were observed as the disease developed. In case of severe infection, the whole leaf died and defoliated (Fig. 2). *Leveillula taurica* was isolated, propagated and re-inoculated on leaves of (60-day old) Ty-20 hybrid. Well developed symptoms were observed (Fig. 3).

Microscopical examination through the lower epidermal strips of infected leaves of Ty – 20 tomato hybrid revealed that branched or unbranched conidiophores arose from the endophytic hyphae, emerged singly or in clusters (uni or bi or more) through the stomata of the lower leaf surface (Alexopoulos, 1952). A conidiophore was observed bearing one conidium. Conidia are long, colourless and cylinderical or pyriform. Its length ranged from 45 to 60 µm with an average of 52.3 µm and its width ranged from 10 to 15 µm with an average of 10.8 µm. Conidiophore lengths ranged from 70 to 250 µm with an average of 143.8 µm and its width ranged from 5 to 10 µm with an average of 8 µm. The mycelium is septate, hyaline with a diameter ranging from 5 to 10 µm with an average of 7.6 µm.

According to the morphological characteristics of conidia, conidiophore and mycelium as well as the different positions of germ tubes during spore germination (Fig. 4), the pathogen in question was identified as *Leveillula taurica* (Lév) Arnaud. Figure (4) also shows the cylinderical and pyriform shapes of the conidia and their basal and lateral germination, which completely agreed with many descriptions (Zwirn,

1943; Yarwood, 1957; Clerk and Ayesu-Offei, 1967; Ahmed, 1969; Natour *et al.*, 1971;El-Fiki and Fawzy, 1987;Cerkauskas *et al.*, 1999 and Haroun, 2002).

As to the spore germination on different substrates , the highest germination was observed on the epidermal strips of tomato leaves (35.2%) followed by germination on cellophane sheet (25.4%) with a significant difference, then on dry slides at 20 % and in water where only 13 % germination occurred (Table 1).

Table 1. Percentage of *L. taurica* conidial germination at 25°C after 24 hr from inoculation using 4 different techniques *in vitro*.

Methods of conidial germination	% of germinated conidia
Epidermal strips	35.2
Cellophane sheet	25.4
Dry slide	20.0
Water	13.0
L.S.D. at 1% level	8.2

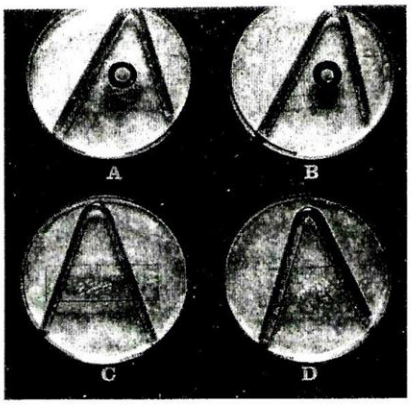
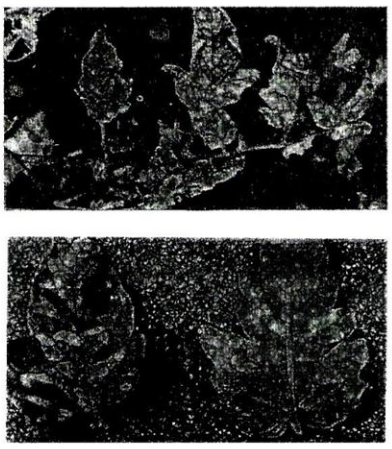


Fig. 1. Four different spore germination techniques for conidial germination of *L. taurica* .
A: Conidia were dispersed on a piece of cellophane sheet which covers a ring containing water.
B: Conidia were dusted on water inside a ring.
C: Conidia were dusted on the upper epidermis of tomato leaf.
D: Conidia were dusted on a dry glass slide.



Upper leaf surface Lower leaf surface
Fig. 2. Natural symptoms of tomato powdery mildew disease in field

Fig. 3. Symptoms of tomato powdery mildew disease on Ty-20 hybrid 15 days after artificial inoculation under greenhouse conditions at 26°C ±2 .

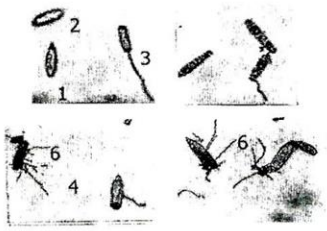


Fig. 4. Conidial shapes and different positions of germ tubes.
1- Pyriform shape. 2- Cylindric shape
3- Basal germination. 4 – Lateral germin.
5-Uni--germ tube. 6-Multi - germ tubes

In this respect, Zwirn (1943),Yarwood (1957), Nour (1958),Clerk and Ayesu-Offei (1967), Zaher (1970), Osman (1977)and Caesar and Clerk (1985) obtained higher percentages of germination when conidia were dusted on dry slides and maintained in moist air than conidia submerged in drops of water and they stated that optimum temperature suitable for conidial germination ranged between 25 and 28°C.

Microscopical examination of the early stages of *L. taurica* spore germination on tomato leaf epidermal layers after 6, 12, 18, 24 hr indicated the following stages :

1) The initial germination of conidia started after 6 hr. Similar result was obtained by Mohamed (1994) and El-Kafrawy(1997).
2) The germ tube elongation started after 12-18 hr. Similar result was obtaind by Caesar and Clerk (1985) and El-Kafrawy, (1997).
3) The positive direction towards stomatal pores occurred within 18-24 hr. (Fig. 5).

Typical symptoms of powdery mildew were realized , seven days later, and conidiophores and conidia were microscopically distinguished (Fig. 6).

In the highly susceptible cv., the fungus grew intercellularly and the mycelium was clearly noticed between the cells of the spongy mesophyl tissue forming many tiny globular haustoria. At the begining, the size of host cells was not altered then, by the development of the disease, a gradual decrease was noticed and the cells became malformed. The cytoplasm and the plastids aggregated around the haustoria. The fungus emerged through the stomata of the lower leaf surface. The conidiophores were long, single or in clusters, septate and bear one mature conidium each (Fig. 7, 8). It was also observed that the numbers of conidiophores and conidia were intensified in the more susceptible host (cv. Strain-B). Few conidiophores and conidia were noticed in the least susceptible cv. Super-strain B (Fig. 9). Moderate numbers of conidiophores and conidia were found in the transverse sections of the moderately susceptible cv. Castle Rock (Fig. 10). Malformation of the mesophyl cells was clearly observed in the infected leaves of the highly susceptible cv. Strain-B. (Fig.11).

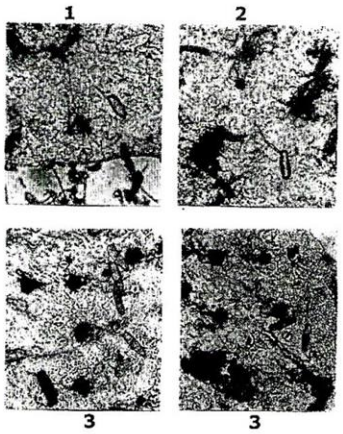


Fig. 5. Conidial germination on tomato upper Leaf surface strips *in vitro*.
1- Initiation of conidial germination after 6 hr.
2-Germ tube elongation after 12-18 hr.
3-Positive direction of germ tube towards the stomata.

Fig. 6. Conidiophores emerging from stomata (200 X). Notice the ovate , clavate and cylindric conidia , seven days after spore dusting .

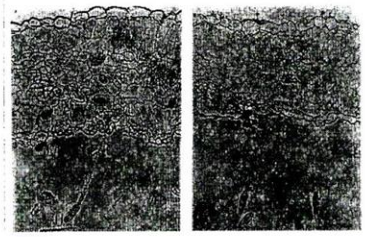


Fig. 7. Long single septate conidiophore bearing one mature pyriform or cylindric conidium . (200 X)

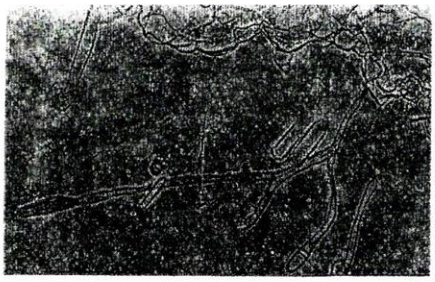


Fig. 8. Single branched conidiophore of *Leveillula taurica*.(200X)

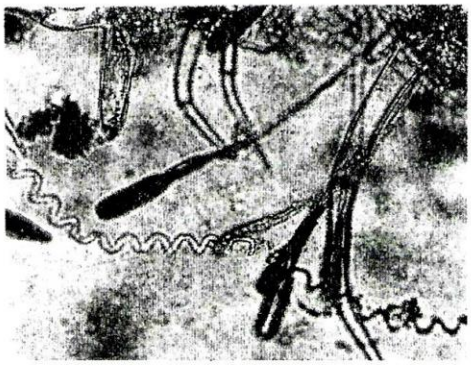
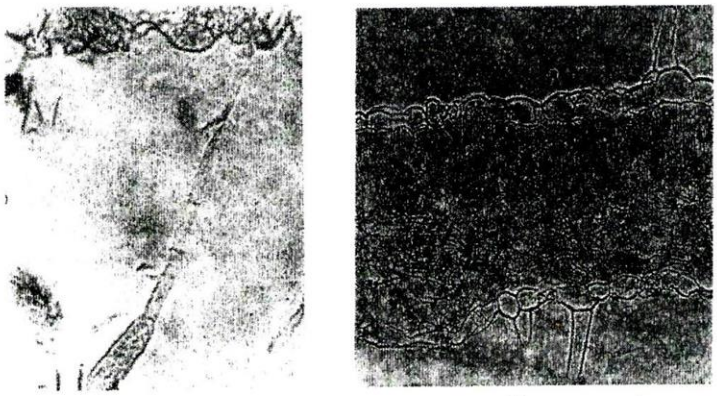
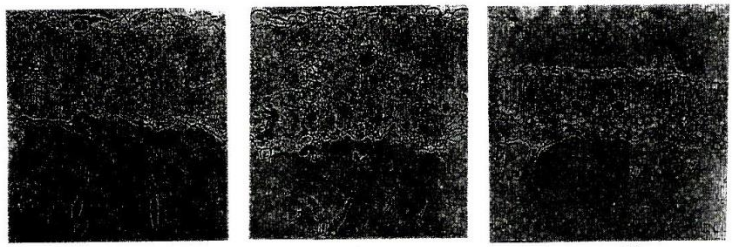


Fig. 8. Conidiophores of *Leveillula taurica* in clusters .(200X)



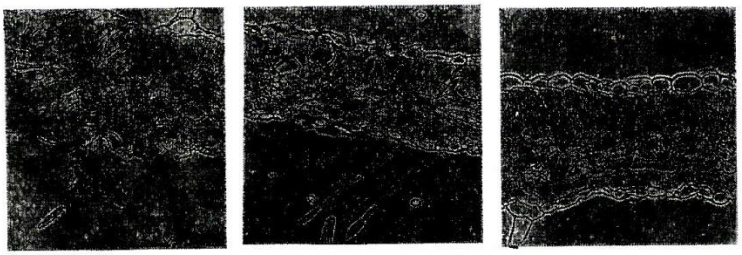
(A) (B)

Fig. 9. Transverse sections in the leaves of the least susceptible tomato cv. Super srain- B .
(A) Infected leaf (400X) . (B) Healthy leaf (200X) .



(A) (B) (C)

Fig. 10. Transverse sections in tomato leaves of the moderately susceptible cv. Castle Rock .
(A and B) : Infected leaves (200 X) . (C) : Healthy leaf (100 X) .



(A) (B) (C)

Fig . 11. Transverse sections in the leaves of the highly susceptible tomato cv . Strain –B (200X) .
(A and B) : Infected leaves . (C) : Healthy leaf

Notice : The malformation of the spongy mesophyl cells due to severe infection

REFERENCES

1. Ahmed, K.G.M. 1969. Studies on powdery mildew of artichoke. M.Sc. Thesis, Dept. of Agric. Bot., Fac. of Agric., Ain Shams Univ.

2. Alexopoulos, C.J. 1952. Introductory Mycology, pp. 244-245, John Willey and Sons, Inc., New York. Chapman and Hall, Ltd., London.

3. Caesar, J.C. and G.C. Clerk. 1985. Germinability of *L. taurica* conidia obtained from water stressed pepper plants. Can J. Bot ., 63 (10): 1681-1684.

4. Cerkauskas, R.F.; J. Brown; G. Ferguson and S. Khosla, 1999. First Report of Powdery Mildew of Greenhouse Pepper Caused by *Leveillula taurica* in Canada. Plant Dis. D-1999-0524-01.N.

5. Cerkauskas, R.F.; L. Leopold and G. Ferguson, 2000. Control of Powdery mildew in greenhouse cucumbers, peppers and tomatoes. Phytopathology , 90 : 512.

6. Ciccarese, F.; M. Amenduni; O. Schiavone and M. Circulli, 1998. Occurrence and inheritance of resistance to powdery mildew (*Oidium lycopersici*) in *Lycopersicon* species. Plant Pathology, 47 (4) : 417-419. (c.f. Rev. Plant Pathol. 1998, 77 (11) 9321.

7. Clerk, G.C. and E.N. Ayesu-Offei , 1967. Conidia and conidial germination in *L. taurica* (Lév) Arn. Ann. Bot.,N.S., 31: 749-754. (c.f. Rev. Appl. Mycol. 47-3-1968).

8. El-Fiki, A.I.I. and R.N. Fawzy.1987. Incidence of *Oidiopsis taurica* on castor bean plant in Egypt. Annals of Agric. Sci. Moshtohor, 25 (2): 807-817.

9. El-Kafrawy, A.A. 1997. Studies on powdery mildew of pepper. Ph.D. Thesis, Fac. of Agric., Minufiya Univ., A.R.E.

10. Georgiev, P., D.Angelov, L. Krasteva and L. Stamova. 1993. Reaction of different tomato varieties to powdery mildew causal agent *S. fuliginea* f. *lycopersicum*. Proceedings of XIIth European meeting on tomato genetics and breeding. Plovdiv, Bulgaria. 27-31 July, 1993, 55-58.

11. Haroun, S.H. 2002. Studies on powdery mildew disease on tomatoes in Egypt. Ph.D. Thesis, Fac. of Agric., Moshtohor, Zagazig Univ., Benha Branch, A.R.E.

12. Huang Caicheng , T. Groot, F. Meijer-Dekerns, R.E. Niks and P.Lindhout 1998. The resistance to powdery mildew (*Oidium lycopersicum* in *Lycopersicon* species is mainly associated with hypersensitive response. Europ . J . Plant Pathol ., 104 (4) 399-407. [c.f. Rev. Plant Pathol., (1998) 77 (11) 9315].

13. Johansen, D.A. 1958. Plant Microtechniques, Mc Graw-Hill Book Co., New York.

14. KangSooWoong, Kwon Jinhyeuk, Shin Wonkyo and Kim Keekyn 1995. Occurrence of powdery mildew on tomato caused by *Oidiopsis taurica* Arnaud- (*Leveillula taurica*) in Korea. Korean J. Plant Pathol., 11 (4): 380-382. (c.f. Rev. Plant Pathol., 74 (1): 445, 1995).

15. Karaseviez, D.M. and T.A. Zitter. 1996. Powdery mildew occurrence on greenhouse tomato plants in New York. Plant Disease , 80 (6): 709. (c.f. Rev. Plant Pathol., 1995, 76 (1), 453.

16. Mohamed, S.A. 1994. Virulence of *L. taurica* (Lév) Arn., on some pepper cultivars and its control. Minufiya Agric. Res., Vol. 19 No. 6 (1): 2883-2902.

17. Natour, R.M. , A.H. El-Behadli and M.G. Majeed. 1971. Occurrence of *L. taurica* in Iraq. Plant Dis. Reptr. 55 (2): 192.

18. Nour, M.A. 1958. Studies on *L. taurica* (Lév) Arn. and other powdery mildews. Trans. Brit. Mycol. Soc., 41, : 17-38.

19. Osman, H.A. 1977. Studies on *L. taurica* the incitant of powdery mildew in Solanaceaous plants. M.Sc. Thesis, Fac. of Agric., Minufiya Univ., A.R.E.

20. Sadasivan, Nair K.R. And A.H. Ellington. 1962. Studies on the germination of conidia spores of powdery mildew of wheat. Phytopathology, 52: 745.

21. Schnathorst, W.C. 1959. Growth of *Erysiphe cichoracearum* on isolated lower epidermis and spongy tissue of lettuce. Phytopathology 49: 304-308.

22. Yarwood, C.E. 1957. Powdery mildews. Botany Review, 32: 235-301.

23. Zaher,E.A.1970.Studies on the powdery mildew incited by *Leveillula taurica*(Lev.) Arn. M.Sc. Thesis, Fac. Agric.,Cairo Univ., Egypt.

24. Zwirn, H.E. 1943. Studies on *Leveillula taurica* (Lév) Arn. Palest. J. Bot. J. Ser. III, pp. 52-53. (c.f. Rev. Appl. Mycol. 24, 476, 1954).

دراسات تشريحية مرضية لأوراق نباتات الطماطم المصابة بالبياض الدقيقى وعلاقة ذلك بقابلية العائل للإصابة

ناجى جورج حنا عوض ، ميشيل فؤاد ابراهيم تادرس
مدحت سعد عبد المجيد

معهد بحوث أمراض النبات - مركز البحوث الزراعية - جيزة – مصر

تـم تعـريف الفطر المسبب لمرض البياض الدقيقى على الطماطم *Leveillula taurica* (Lév.) Arnaud تـبعاً للأعراض التى يحدثها الفطر وصفاته المورفولوجية والخصائص المميزة لحوامله وجراثيمه الكونيدية .

تُنبت الجراثيم الكونيدية غالباً إما عند قاعدتها أو جوانبها مكونةً أنابيب إنبات مفردة أو عديدة ، كما لوحـظ أن إنبات الجراثيم الكونيدية على سلوخ أوراق الطماطم والمحاطة برطوبة عالية يكون جيداً عنه فى حالة وضعها فى الماء الحر أو الظروف الجافة .

يعتمد تكوين عدد الحوامل الكونيدية ومدى تفرعها على قابلية صنف الطماطم للأصابة حيث لوحظ تكوين عدد قليل من الحوامل الكونيديةالفردية وغير المتفرعة على أقل الأصناف إصا(سوبر سترين- بى) كمـا تكوّن عدد كبير منها قد يتفرع بعضها على الصنف (سترين - ب) ذو القابلية العالية للاصابة ويتكون عددمتوسـط مـن الجراثـيم والحوامل الكونيدية على الأسطح السفلى لأوراق الصنف (كاسل روك) متوسط القابلية للاصابة .

أوضـح الفحص الميكروسكوبى لمراحل إنبات الجراثيم الكونيدية أن أنابيب الانبات تبدأ فى الظهور تحت الظـروف الدافـئة الرطبة بعد ٦ ساعات من العدوى وتستمر فى استطالتها خلال١٢-٢٤ ساعة متجهة نحو ثغـور السطح العلوى لأوراق العائل ، هذا وتظهر الأعراض المُشخصة للمرض على أوراق نبات الطماطم بعد ٧ أيام من الحقن تحت ظروف الصوبة على درجة حرارة ٢٥ ٦ م ± ٢ متزامنة فى ذلك مع أوراق أخرى تم نزعها ثم حقنها لدراسة مراحل الانبات الأولى معملياً .

أوضـحت الدراسة التشريحية لقطاعات الأوراق المصابة للأصناف المذكورة نمو ميسليوم الطفيل بيـن خلايـا النسـيج الميزوفـيلى الأسفنجى مكوناً عدداً كبيراً من الممصات الدقيقة داخلها ، ويتقدم حدوث الاصـابة لوحـظ إنكمـاش تدريجـى فـى حجم هذه الخلايا وتشوهها كما لوحظ تجمع سيتوبلازم الخلايا وبلاستيداتها الخضراء حول هذه الممصات .

لوحـظ أيضـاً خـروج الحوامل الكونيدية للفطر من ثغور السطح السفلى ووجدت مقسمة ومفردة وطويلة وغالباً فى تجمعات وتحمل جرثومة كونيدية واحدة فى أكثر الأصناف أصابةً، وبصفة عامة تبين أن عدد الحوامل الكونيدية يزداد كلما زادت قابلية الصنف للإصابة وحدوث تشوه شديد لخلايا ميزوفيل أكثرفى الأصناف قابلية للإصابة (الصنف سترين – بي) .