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CLOVER PHYLLODY DISEASE IN JUGOSLAVIA

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Introduction

Clover phyllody belongs to the yellows-type diseases. Among these diseases stolbur was particularly well examined in Jugoslavia (Panjan 1958, Klindić and Buturović 1959, Miličić 1964, Aleksić et al. 1967, Plavšić-Banjac 1967). All these cited papers were published at the time when yellows were thought to be virus diseases. Yellows were classified as virus diseases because their characteristics were similar to those of viruses, but viruses could not be found in the infected plants.

The first data about the occurrence of other yellows-type diseases in Jugoslavia were also established at that time. Panjan and Grancini (Bos et al., 1965) noticed the virescence of Jugoslav white clovers. Later Blattný (1970) found white clovers infected with clover phyllody in environs of Maribor. As this disease was not investigated in Jugoslavia then, we have undertaken a series of investigations, which are published in this paper.

In 1967 there was an important turn in the knowledge of the yellows aetiology. Doi et al. found that the yellows-type diseases: mulberry dwarf, potato witches' broom and aster yellows were not caused by viruses but by microorganisms with cellular organization, i. e. by mycoplasma-like organisms (MLO). There-upon numerous investigations of other yellows-type diseases followed. Maramorosch et al. (1970) stated that from 1967 to 1970 more than 40 plant diseases were found to be caused by MLO. Among other authors Panjan et al. (1970) found that a potato disease with typical symptoms of stolbur was caused by MLO. Plavšić-Banjac and Maramorosch (1973) established that lethal yellowing of coconut palms was provoked by MLO. Clover phyllody was also investigated in this sense, and so Maillet et al. (1968) and Sinha et al. (1969) proved the presence of MLO in phloem elements.

Material and Method

Naturally infected white clovers (*Trifolium repens* L.) with the symptoms of flower greening and phyllody were found in relatively large areas in Zagreb, Sarajevo and in the surroundings of Sarajevo.

For electron microscope investigations strips of tissue were fixed in 1% glutaraldehyde in cacodylate buffer and postfixed for 2 h in 1% osmium tetroxide. After fixation samples of tissue were dehydrated in ethanol series and embedded in Araldite. The ultrathin section were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I.

Floral Abnormalities

Naturally infected white clovers show evident changes on flowers and inflorescences (Figs. 1 and 2). All flower parts of the diseased clovers are changed, and particularly remarkable are the abnormalities of the calyx and pistil. The degree of calyx abnormalities varies from a slight enlargement to the complete transformation into leaves, while the pistil is mostly transformed into a relatively large follicle-like structure or a leaflet.

The calyx of a normal flower is made of five sepals grown together into a tube with five unequal teeth (Fig. 3 a). Unlike the normal calyx, the calyx teeth of affected clovers become leaf-like. Each tooth transforms into a leaf-like structure with or without a developed petiole (Figs. 2 b, c, d; 3 b).

The corolla of healthy plants is made of five petals of different forms. The largest petal is called the standard (vexillum), two side ones are wings (alae) and two other petals, more or less grown together at their lower margins, make a keel (carina). The corolla of the infected clovers is almost completely dwarfed and can be noticed only when the calyx tube is removed.

The stamens are ten in number; nine of them have grown together by filaments into a tube, and one is free. Changes on stamens of the diseased clovers are manifested by shortened filament and tube, formed by coalesced filaments.

The pistil is made of one carpel, and the ovary is superior. The fruit is a small, non typical pod. In the flowers of diseased clovers the greatest changes occur in gynoecium. The pistil is hypertrophied. The enlargement of the ovary can be very extensive so that the ovary (Figs. 2 a, b; 3 c) can reach the length of about 25 mm. The enlarged ovary opens partially along the ventral suture as follicle so that leaf-like ovules are visible in it (Fig. 2 c, d). The pistil transformation proceeds by complete opening of its ventral suture at the margins of which leaf-like structures represent metamorphosed ovules (Fig. 3 e).

Depending on the changes in the calyx, corolla, stamens and pistil, the flower loses its morphological characteristics and becomes virescent and more or less leaf-like. In all floral abnormalities, calyx can be either slightly enlarged or completely leaf-like. It embraces the pistil which is either leaf-like or green and enlarged. The calyx and its teeth are frequently prolonged. The teeth are often metamorphosed into five simple leaves containing only one leaflet (Fig. 1 b-d) but sometimes con-

sisting of three leaflets. In some flowers the calyces, corollas, and stamens are normally developed, only the pistil grows up into a large pod as long as the standard. Such floral abnormalities are not frequent.

An important form of metamorphosis is flower proliferation. New buds sprout in the axil of the calyx and develop into leaf-like structures (Fig. 2 g, h).

Healthy stems of white clover bear numerous white flowers gathered into head-like inflorescences. Flowers on short, erect stalks are geotropically negative (Fig. 1 a). During the development of the flower in the fruit and seed, flower stalks become more elongate, they bend down towards the ground so that the withering flowers have a positive geotropic orientation (Fig. 1 b).

Infected clovers form rather big, green inflorescences with various floral abnormalities (Fig. 1 c, d, e). Inflorescences can vary both in structure and in size, so that small head-like inflorescences with dense green metamorphosed flowers on short flower stalks can be found too. Metamorphosed flowers have very long and always erect flower stalks. They do not change the nature of geotropic reaction, which is a characteristic of normal flowers of white clover. Erect growth of flowers is also a symptom of stolbur infection and of some other yellows-type diseases.

Inflorescences mostly consist either of flowers with leaf-like metamorphosed parts of pistil or of flowers with enlarged follicle-like pods (Fig. 1 d, c), or of flowers with leaf-like transformation of calyx (Fig. 1 e). All flowers of one and the same inflorescence usually show the same sort of anomaly. For instance, in Fig. 1 c all flowers have enlarged and green pods, retaining their colour for a long time, in Fig. 1 d all flowers have the form of erect leaves, and inFig. 1 e all flower parts have a well developed calyx whose teeth have the form of laminae. All inflorescences of the same plant often show the same type of anomaly, but there are also exceptions to this rule. On the basis of observations made in various seasons, it seems that the type of floral abnormalities varies and some types prevail in a certain season. It would be interesting to continue investigations and experiments to see whether different types of deformation are caused by external factors.

In analysing the pistil, we could notice deformed ovules in the form of leaflets at the carpel margins. No doubt such ovules were sterile. The degree of sterility was not examined. On the basis of investigations done so far, it seems to be very high.

Electron Microscopy

Electron-microscopy investigations discovered numerous MLO in the phloem elements of the infected plants (Fig. 4 and 5). Numerous polymorphic microorganisms ranging from 125 to 825 nm in diameter were wrapped in a single unit membrane. Fibrils which can be seen in the central part of the cell are supposed to be DNA. Unlike the centre, the peripheral layer is granulated. Particles of the granulated layer are ribosome-like. MLO have not been observed in the healthy control plants.

Discussion

Changes in the flowers and in inflorescences of naturally infected white clovers in Jugoslavia are similar to those caused by clover phyllody described earlier in England (Frazier et al., 1957), in Czechoslovakia (Mišiga et al., 1960), in the Netherlands and Italy (Bos et al., 1965).

White clover is a host plant to some other yellows, such as clover dwarf, parastolbur (Valenta et al., 1963), witches' broom (Frazier et al., 1957) and stolbur (Helms, 1962; Valenta et al., 1961).

Floral abnormalities examined here largely correspond to the anomalies examined in detail by Miličić (1964) on tobacco affected by stolbur. This implies particularly the corolla and stamens which are quite stunted in tobacco plants too. In tobacco, ovules also transform into leaf-like structures which leads to sterility of infected plants.

During our investigations the presence of mycoplasma-like organisms was proved in phloem elements of naturally infected white clovers. In size, polymorphic shape and structure these microorganisms strongly resemble the MLO found in phloem elements of plants infected by clover phyllody by Maillet et al. (1968) and Sinha et al. (1969)

MLO were also found in vector tissue (Sinha et al., 1972), and their disappearance was proved after the therapy of infected plants and vector *Macrosteles fascifrons* with terramycin. Sinha (1974) discovered MLO — which did not differ from the ones described earlier in the sections of infected plants — in purified aster (*Callistephus chinensis* Nees.) preparations infected by clover phyllody.

Fig. 1. *Trifolium repens* L. — *a* Inflorescence of healthy plant. *b* Inflorescence of healthy plant after blooming. *c* Infected inflorescence with metamorphosed follicle-like pistils. *d, e* Inflorescence with leaf-like metamorphosed flowers.

Sl. 1. *Trifolium repens* L. — *a* Cvat zdrave biljke. *b* Ocvala inflorescencija zdrave biljke *c* Inficirana inflorescencija sa mjehurasto metamorfoziranim tučkom. *d, e* Cvat sa listoliko metamorfoziranim cvjetovima.

Fig. 2. *Trifolium repens* L. — *a* Follicle-like metamorphose of pistils with normally developed calyx. *b* Follicle-like pistils with leaf-like calyx. *c, d* Follicle-ike deformations of pistils with leaf-like ovules emerging from carpel. *e, f* Leaf-like transformation of pistil. *g, h* Proliferation of leaf-like metamorphosed flower.

Sl. 2. *Trifolium repens L.* — *a* Mjehurasta metamorfoza cvijeta sa normalno razvijenom čaškom. *b* Cvijet mjehurast sa filoidnom čaškom. *c, d* Mjehurasta deformacija tučka sa listolikim sjemenim zamecima koji izbijaju iz karpelnog lista. *e, f* Listolika transformacija tučka. *g, h* Proliferacija listoliko metamorfoziranog cvijeta.

Fig. 3. *Trifolium repens* L. — *a* Flower of healthy clover, *b* Leaf-like transformation of calyx. *c* Leaf-like transformation of calyx with pistil transformed in follicle-like formation. *d* Leaf-like metamorphose of postil and calyx. *e* Leaf-like carpel with leaf-like ovules.

Sl. 3. *Trifolium repens* L. — *a* Cvijet zdrave djeteline. *b* Listolika transformacija čaške. *c* Listolika transformacija čaške sa tučkom transformiranim u tvorevinu sličnu mjehuru. *d* Listolika metamorfoza tučka i čaške. *e* Listoliki plodni list sa listolikim sjemenim zamecima.

Fig. 4. i 5. Sieve tube of clover naturally infected by mycoplasma-like organisms (marked by arrows). ET elementary bodies. Magnification 40,000 : 1.

Sl. 4. i 5. Sitasta cijev prirodno inficirane djeteline sa organizmima sličnim mikoplazmi (označeni strelicama). ET elementarna tijela. Povećanje 40,000 : 1.

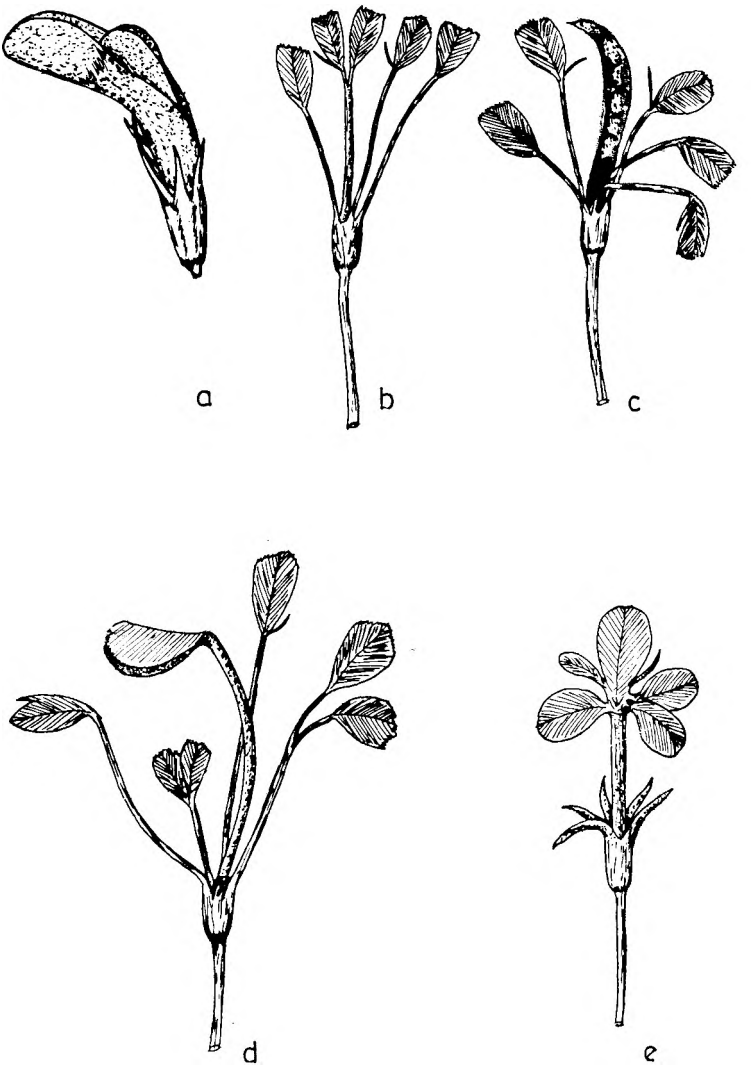

Fig. 3. — Sl. 3.

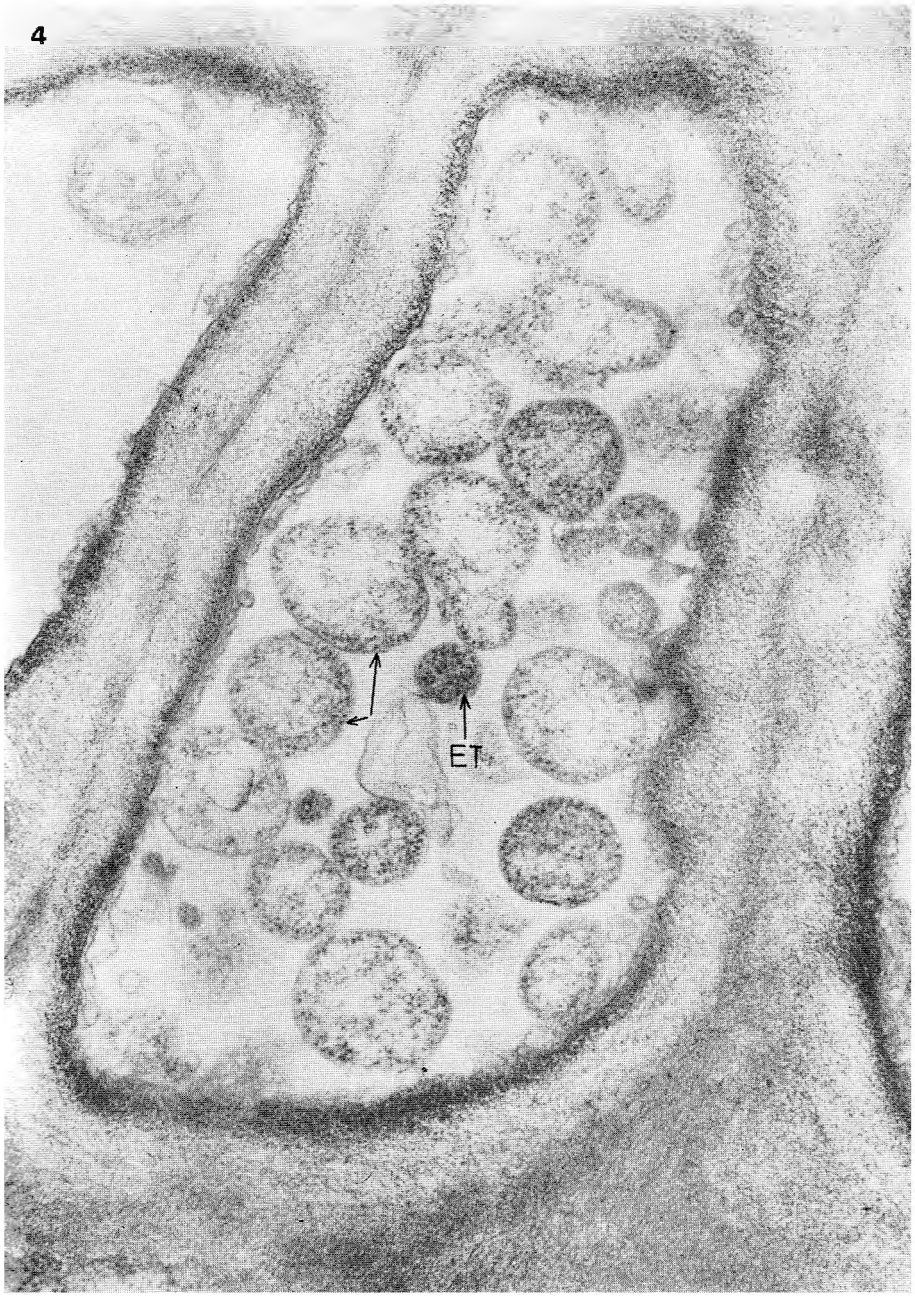

Fig. 4. — Sl. 4.

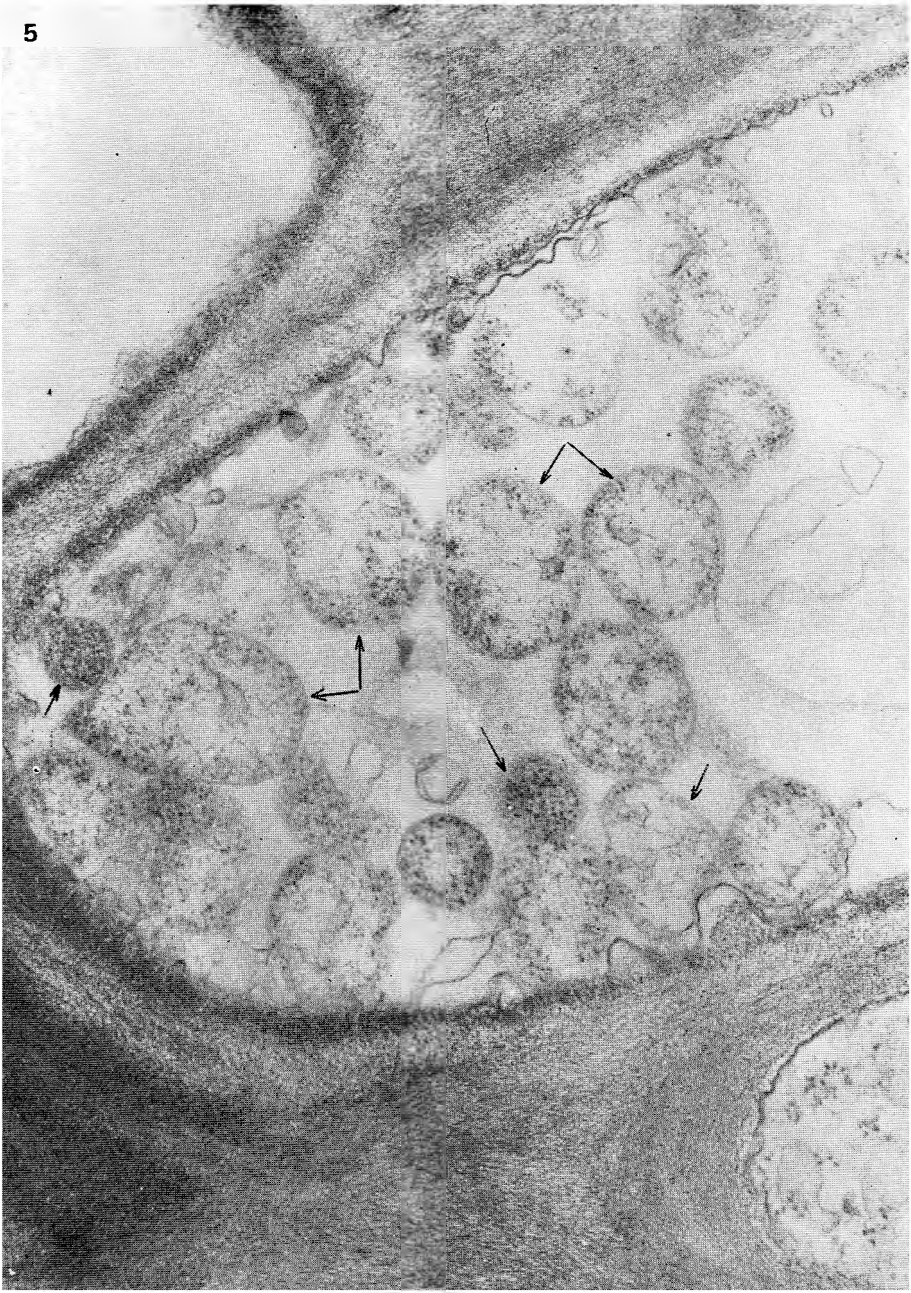

Fig. 5. — Sl. 5.

Summary

White clovers (*Trifolium repens* L.) with symptoms of flower greening, with virescent inflorescences and with yellowing particularly at the marginal parts of leaves, were found in relatively large areas in Zagreb and Sarajevo. These symptoms are characteristic of yellows-type disease which is called clover phyllody and is caused by mycoplasma-like organisms (MLO).

The structure of abnormal flowers and of inflorescences is described. It has been established that changes affect particularly the calyx and the ovary which are often transformed into leaf-like organs. Even ovules can become green. The greening of the ovary and ovules causes sterility. Whether sterility of plants is total or partial, remains unknown.

Numerous polymorphic MLO of characteristic form and structure were proved by electron microscope in the phloem elements of infected plants. In the healthy control plants of white clovers, MLO were not observed. Therefore, the described abnormalities of white clover flower are caused by MLO.

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SADRŽAJ

BOLEST FILODIJA DJETELINE (*CLOVER PHYLLODY*) U JUGOSLAVIJI

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Prirodno inficirane bijele djeteline (*Trifolium repens* L.) sa simptomima ozelenjavanja i prolistalim inflorescencijama nađene su na većim površinama u Zagrebu, Sarajevu i okolici Sarajeva.

Panjan i Grancini (Bos i sur., 1965) prvi su primijetili na jugoslavenskoj djetelini prolistale i ozelenjele inflorescencije. Bolest tom prilikom u Jugoslaviji nije bila istraživana.

Inficirana djetelina pokazuje uočljive promjene u području cvijeta i inflorescencije (sl. 1 i 2). Svi dijelovi cvijeta bolesne djeteline znatno se promijene, a naročito su izražene deformacije čaške i tučka. Stupanj deformacije čaške varira od neznatnog povećanja do potpune transformacije u listove (sl. 3 b). Tučak hipertrofira. Povećavanjem plodnice i vrata tučka nastaje tvorba koja podsjeća na oveći mjehur (sl. 2 a, b; 3 c). Daljna transformacija tučka je listolika, a nastaje otvaranjem ventralnog šava plodnog (karpelnog) lista na čijem rubu leže listoliki metamorfozirani sjemeni zameci (sl. 3 e). Vjenčić bolesnih biljaka gotovo je posve zakržljao i može se primijetiti tek kad se otvori cijev čaške. Promjene na prašnicima izražene su u skraćivanju njihovih filamenata, odnosno cijevi nastale sraštavanjem filamenata.

Uzevši u obzir sve anomalije cvijeta možemo kazati da čaška može biti neznatno povećana do potpuno listolika; ona obavija listoliki ili jako povećani tučak koji nalikuje na mjehur jer se otvara samo na trbušnom šavu. Često je čaška produžena u pet listolikih tvorbi, a tučak mjehurastog oblika ne prelazi polovinu dužine čaške te se između njenih listolikih tvorevina gotovo i na primjećuje (sl. 1 e). Značajan oblik modifikacije cvijeta inficiranih djetelina je proliferacija cvijeta. Novi pupovi se javljaju u pazušcu lista čaške i razvijaju se u listolike tvorevine (sl. 2 g, h).

Inficirana djetelina formira izrazito krupne, zelene inflorescencije se različitim deformacijama cvijeta (sl. 1 c, d, e). Metamorfozirani cvjetovi u inflorescenciji su na izrazito dugim cvjetnim stapkama i ne mijenjaju karakter geotropne reakcije što je inače karakteristično za normalno građen cvijet bijele djeteline. Kao što postoje razni tipovi pozelenjelih cvjetova, tako isto postoje i razne vrste anomalnih inflorescencija. Cvat čine cvjetovi sa listolikom ili »mjehurastom« transformacijom tučka (sl. 1 c, d), ili sa listolikom deformacijom čaške (sl. 1 e). Obično svi cvjetovi jedne inflorescencije pokazuju istu vrstu anomalije. Opisane morfološke promjene cvijeta i cvati te pojava žućenja rubnih dijelova lista karakteristični su simptomi oboljenja filodija djeteline (clover phyllody).

U floemskim elementima prirodno inficirane bijele djeteline elektronsko-mikroskopskim istraživanjima otkriveni su brojni organizmi karakterističnog oblika i strukture slične mikroplazmama. Brojni polimorfni mikroorganizmi veličine od 125—825 nm u promjeru obavijeni su elementarnom membranom (sl. 4). Centralni dio stanice sa nitima sličnim DNK okružen je granuliranim slojem citoplazme. Čestice granuliranog sloja slične su ribosomima. U kontrolnim zdravim biljkama djeteline mikroorganizmi nisu nađeni. Prema tome, opisane deformacije cvijeta bijele djeteline vjerovatno su izazvane infekcijom od organizama sličnih mikoplazmi.

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