Outbreak of a New Phytoplasma Disease of Onion in the Czech Republic*

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Abstract

POLÁK J., JOKEŠ M. (2000): **Outbreak of a new phytoplasma disease of onion in the Czech Republic**. Plant Protect. Sci., **36**: 128–131.

In 1999, split vegetation tops in onions, cv. Štutgartská, grown in the surroundings of Prague, Bohemia, were observed. Such plants developed two or more bulbs from one originally planted. In addition, irregular and asymmetrical drying of the apical parts of outer skirts and in some cases scaling of the outer parts of divided bulbs occurred. Such divided bulbs suffered substantially higher damage during storage in winter; premature budding and growth of individual parts was frequent. Further splitting of the vegetation tops into two to five parts and followed by premature budding was observed in individual parts of divided bulbs. Ultrathin sections were prepared from roots and leaves of divided bulbs. Phytoplasma bodies were found in tissues of both roots and leaves. Such bodies were not found in roots and leaves of healthy looking and undivided bulbs

Key words: phytoplasma disease; onion; vegetation top splitting; ultrathin section; roots; electron microscopy; phytoplasma bodies

The yellows disease of onion was first described in the U.S.A. (LARSON & WALKER 1944). Aster yellows virus was demonstrated to be the causal agent at that time. Later DOI *et al.* (1967) proved that phytoplasmas are responsible for aster yellows. The yellows disease of onion was manifested by virescences. Infected plants were chlorotic, the leaves became thin and bolted, sometimes flattened and rugose. The disease was found in the former Czechoslovakia and later described in detail by NOVÁK (1953, 1959). Studies on transmission by the leafhoppers *Macrosteles laevis* Rib. were carried out in Poland by KOCHMAN & KSIAŻEK (1964). In tissues of onions with symptoms of proliferation and aspermy, phytoplasma bodies of 80 to 600 nm in size were found in Roumania by PETRE & PLOAIE (1973). They transmitted the disease from onion to *Vinca rosea* and *Chrysanthemum carinatum* by the dodder *Cuscuta campestris.* Recently were proved the occurrence and properties of phytoplasmas on union in Japan (SHIOMI *et al.* 1996; KUBOYAMA *et al.* 1998; NISHIMURA *et al.* 1998). Studies on molecular identification of phytoplasmas infecting onion were published from Italy (VIBIO *et al.* 1995).

A disease of onion with potential phytoplasma etiology was observed in the surroundings of Prague in 1999. The symptoms of this disease differed considerably from those described earlier to be caused by aster yellows. Results of our preliminary studies on the etiology of the disease are the subject of our present communication.

MATERIAL AND METHODS

Plant Material. Splitting of the vegetation tops and development of two to five bulbs from one originally planted was observed in the onion cv. Štutgardská (Fig. 1).

Fig. 1. Split bulb of onion infected with phytoplasma

*This work was supported by Research grant of Ministry of Agriculture M01/00:06.

Fig. 2. Mild yellowing and rugosity of leaves, mild dwarfing or bushy stunt of plants developed from a split bulb infected with phytoplasma

The disease was also manifested by lighter colour and rugosity of leaves, mild dwarfing or bushy stunt (Fig. 2). Bushy appearance was caused by growth of eight vegetation tops from one doubled bulb. Symptoms of amplification of bulbs occurred in over half of the onion plants. After harvest of the bulbs, irregular and asymetrical drying of the apical parts of outer skirts and in some cases scaling of the outer sides of divided bulbs appeared. In individual parts of divided bulbs a further division of the vegetation top into two to five parts occurred. After planting of such bulbs, single plants developed from each vegetation top, but the number of inflorescences was lower than the number of developed plants. For instance, from a bulb divided into two parts altogether eight plants developed, of which only five produced flower stalks. The flowers did not reveal any virescences or other symptoms.

Electron Microscopy. Ultrathin sections were prepared from roots and leaves newly expanded from divided bulbs. In the period of flowering also flowers and flower stalks were used for sectioning. As a control, samples of roots, leaves and flowers of healthy plants were used. Pieces of sampled tissues about $1 mm^3$ in size were fixed for 90 min in 6.25% solution of glutaraldehyde in phosphate buffer pH 7.0, for 60 min postfixed in 1% osmium tetroxide and washed for 90 min with phosphate buffer. After dehydration the specimens were embedded in a four-component resin "Resin KIT" and hardened for 24 h at 60°C. The ultramicrotome Ultracut E was used for sectioning. Specimens were then stained with uranyl acetate and lead citrate and examined with a Philips 208 S electron microscope.

RESULTS AND DISCUSSION

Phytoplasma bodies were found in ultrathin sections prepared from roots and leaves of diseased onions. Large numbers of them were observed in cells of the root tissue (Fig. 4). Their size varied from 400 to 1,200 nm (detail, Fig. 5). The bodies exhibited typical phytoplasma structure with a double cell wall. In leaf cells and sieve tubes rather isolated phytoplasma bodies were found (Fig. 6 and 7). Such bodies were not found in roots and leaves of healthy, undivided onions.

Divided and prematurely budded bulbs flowered later than healthy ones. In 2000 (with an extremely warm spring) they flowered by the end of July and at the beginning of August. Inflorescences were smaller but normal without any symptoms of virescence, phyllody, proliferation and aspermy (Fig. 3) described by NOVÁK (1959) and by PETRE & PLOAIE (1973). Both in flowers and flower stalks we failed to find typical phytoplasma bodies.

We assume that the new phytoplasma disease of onion found in the Czech Republic differs from aster yellows and from the earlier described yellows disease of onion, including the very recent finding on *Allium altyncolicum* (NAVRÁTIL *et al.* 2000). Our assumption is supported by the fact that the highest concentration of phytoplasma bodies occurred in the roots of diseased onions while none were found in the flowers. In contrast, the occurrence of phytoplasma bodies in flowers and flower organs is typical for the disease caused by aster yellows which is manifested by virescence, phyllody and proliferation in flowers (e.g., POLÁK & JOKEŠ 1997). In the new disease, the splitting of vegetation tops is followed by poorer storability of divided bulbs, by premature budding and growth of divided parts.

While the phytoplasma etiology of the new disease of onion was substantiated by numerous bodies in phloem

Fig. 3. Eight grown up plants infected with phytoplasma; five of them developed flower stalks

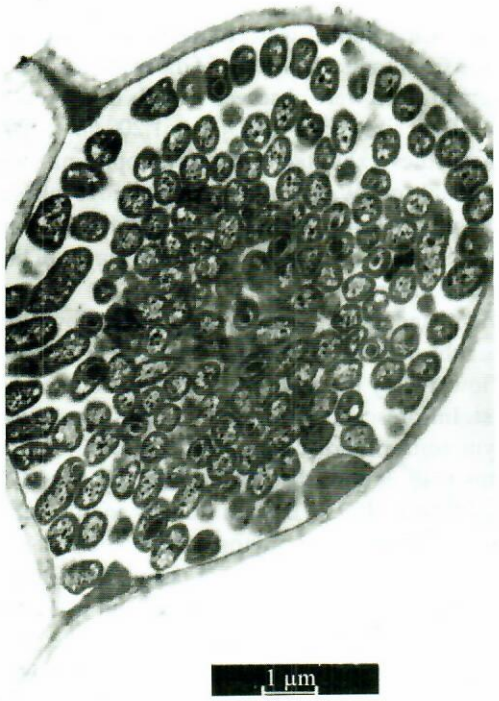

Fig. 4. Root cell of onion filled with phytoplasma bodies. Direct magnification 6,300×, total magnification 14,500×

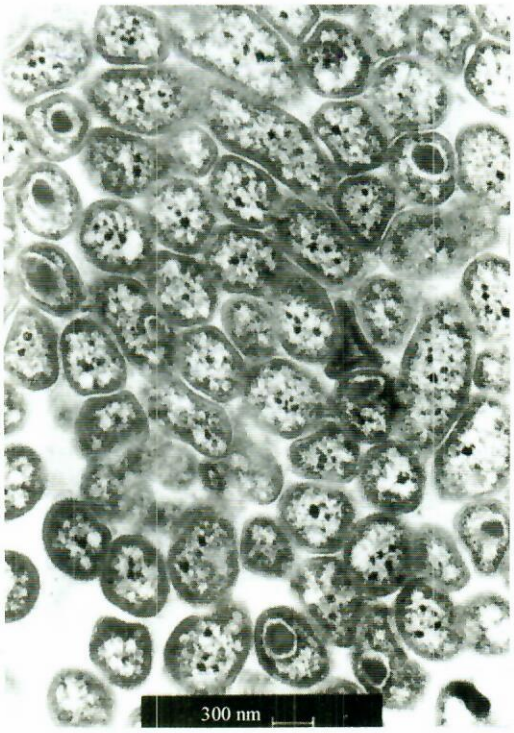

Fig. 5. Detail of a phytoplasma body in a root cell of onion. Direct magnification 16,000×, total magnification 36,800×

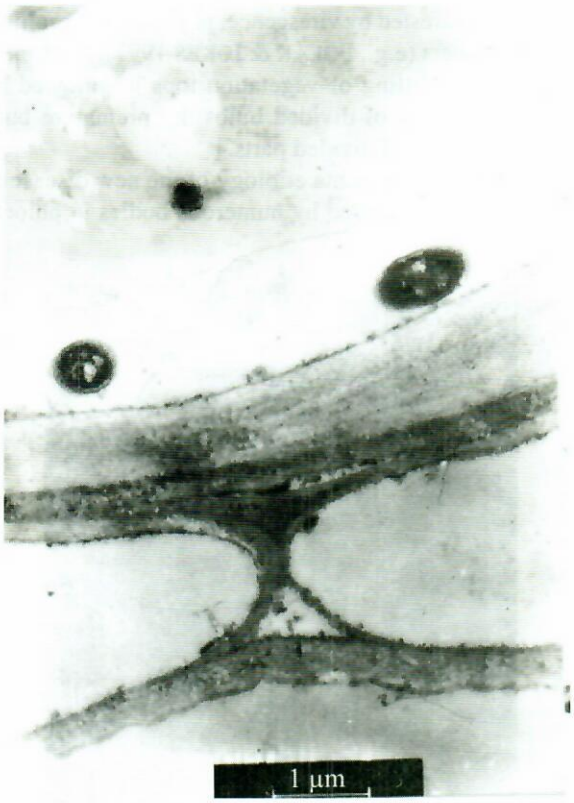

Fig. 6. Detail of a leaf sieve tube with two phytoplasma bodies. Direct magnification 10,000×, total magnification 23,000×

tissue of roots and leaves of diseased onions, an exact determination of the causal agent of the disease should be achieved by molecular methods.

Yellows diseases caused by phytoplasma, including potato stolbur, occurred relatively often in warmer regions of Europe and also in Czechoslovakia in the fifties and sixties. In the seventies and eighties they considerably receded. Probably in connection with the warming trend in the nineties the occurrence of these diseases increased again; this applies e.g. in the Czech Republic to phytoplasma in winter rape (POLÁK & JOKEŠ 1997; BERTACCINI *et al.* 1998), leek (BERTACCINI *et al.* 1999), strawberry (FRÁNOVÁ-HONETŠLÉGROVÁ *et al.* 1996) and fruit trees (NAVRÁTIL *et al.* 1998).

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Received for publication August 25, 2000
Accepted for publication November 24, 2000

Souhrn

POLÁK J., JOKEŠ M. (2000): **Výskyt nového fytoplasmatického onemocnění cibule v České republice.** Plant Protect. Sci., **36**: 128–131.

V roce 1999 bylo v Čechách (v okolí Prahy) pozorováno dělení vegetačního vrcholu u odrůdy cibule Štutgartská a vytváření dvou a více cibulí (zpravidla dvou až pěti) z původně jedné cibule – sazečky. Kromě toho docházelo po sklizni k nepravidelnému a asymetrickému zasychání apikální části vnějších suknic, v některých případech k šupinovitosti na vnější straně rozdělených cibulí. Skladovatelnost takovýchto rozdělených cibulí byla podstatně zhoršena a během zimního období docházelo často k předčasnému rašení a růstu jednotlivých částí. V jednotlivých částech rozdělené cibule došlo k dalšímu dělení vegetačního vrcholu na dvě až pět částí, které předčasně vyrašily. Deformace, znetvoření a virescence květenství ani proliferace jednotlivých květů a přeměna květů v listy nebo v nové zelené rostlinky s cibulkami nebyly zjištěny. Absence příznaků na květech odlišuje tuto novou chorobu cibule od již dříve na cibuli popsané žloutenky aster. Z kořenů a listů rašících rozdělených cibulí byly připraveny ultratenké řezy. V pletivu těchto kořenů byly zjištěny částice podobné fytoplazmě. V kořenech a listech zdravé, nedělené cibule takovéto částice zjištěny nebyly.

Klíčová slova: fytoplazmové onemocnění; cibule; dělení vegetačního vrcholu; ultratenké řezy; kořeny; listy; elektronová mikroskopie; fytoplazmatická tělíska

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