First report of a yellows disease of German statice (*Goniolimon tataricum*) in Canada caused by a phytoplasma

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Symptoms of leaf reddening, chlorosis and flower phyllody of German statice (*Goniolimon tataricum* (L.) Boiss), caused by a phytoplasma, were found in plantings at Olds and Lac La Biche, Alberta in 1994 and 1995. Transmission electron microscopy showed that the phytoplasma bodies ranged in size from 0.37-0.82 x 0.32-0.53 μm (mean = 0.49 x 0.42 μm), were oval to spherical, lacked cell walls, and were surrounded by single membranes. Chloroplasts in the diseased plants appeared to be swollen or degenerated. Survey results indicated that the economic impact of this disease on the commercial production of statice for floriculture use could be significant. The mode of disease transmission under natural conditions is discussed. This is the first report of a phytoplasma disease of German statice in Canada.

Can. Plant Dis. Survey, 76:1, 15-20, 1996.

Les symptômes de rougissure, chlorose et faillit de *Goniolimon tataricum* (L.) Boiss, causés par le phytoplasme, ont été retrouvés dans des plantations à Olds et Lac La Biche, en Alberta, en 1994 et 1995. La microscopie électronique à balayage a révélé que les particules de phytoplasme variaient pour ce qui est de la grosseur entre 0,37-0,82 x 0,32-0,53 μm (moyenne = 0,49 x 0,42 μm) et avaient une forme sphérique ou ovale. Il n'y avait pas de parois cellulaires et une membrane simple les enveloppait. Le chloroplaste dans les plantes atteintes de maladies semblait être enflé ou dégénéré. Les résultats d'une étude ont démontré que les répercussions économiques de cette maladie pour ce qui est de la production commerciale des statices en floriculture pourraient être importantes. Des discussions sont en cours sur le mode de transmission de la maladie dans des conditions naturelles. Il s'agit du premier rapport concernant le phytoplasme chez les statices au Canada.

Introduction

German statice (*Goniolimon tataricum* (L.) Boiss) (syns. *Limonium tataricum* (L.) Mill; *Statice tatarica* L.), a perennial plant grown for cut flowers (Fig. 1) or dry bouquets (Shillo and Zamski, 1985; Godwin, 1990), has gradually gained popularity in Alberta. Statice plants are slow to establish and require two or three years to achieve optimum flowering (Godwin, 1990). Healthy plants produce panicles of spikelets with two to six small blooms and each flower has a funnel-shaped, five-lobed white calyx (Fig. 1). Symptoms resembling those caused by a phytoplasma (mycoplasma-like organism, MLO) infection were observed on Alberta field-grown statice plants at Olds in 1994 and at Lac La Biche in 1995. The most obvious symptoms on diseased plants were leaf reddening and yellowing (Fig. 2), plant stunting (Fig. 3), and abnormal flower development (Fig. 4). Several diseases of *G. tataricum* have been reported in Europe, including fungal diseases caused by *Fusarium* spp. and *Rhizoctonia solani* in Poland (Skrzypczak, 1992) and by *Peronospora statices* in Hungary (Szabo and Viranyi, 1990). In addition, Novak *et al.* (1980) reported 25% infection of statice plants with tomato bushy stunt virus in the Czech Republic and a MLO disease of *G. tataricum* was reported in Germany (Marwitz *et al.* 1986). The objectives of this study were to determine the etiology and incidence of a yellows disease in German statice field plantings in Alberta.

Materials and methods

Determination of disease incidence

In June, 1993 and 1994, German statice seedlings were transplanted into field plots at Olds in six, 90 m rows with 1.0 m between rows and 30 cm between plants within the rows, and at Lac La Biche, the statice was planted in 45 m rows at the same spacing used at Olds. All plants at Olds were surveyed visually for yellows symptoms in July, 1994. In September, 1995, plants at both locations were surveyed for yellows. Percentage disease incidence values were determined by dividing the number of diseased plants by the total number of plants surveyed in each field.

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Accepted for publication January 3, 1996.

Isolation of bacteria and fungi from diseased plants

Leaf tissues from affected plants were cut into 5-mm-long pieces, immersed in 1% sodium hypochlorite for 3 min and rinsed three times in sterile distilled water. Leaf pieces were transferred to petri dishes containing potato dextrose or nutrient agar media; the plates were incubated at 22°C in darkness for one week, then examined for microbial growth.

Tissue examination by TEM

Specimens obtained from leaf midribs, young stems and flowers with phyllody symptoms were cut into 2 mm sections. These materials were immediately immersed in a fixative containing 5% glutaraldehyde and 4% formaldehyde in a 0.1 M cacodylate buffer at pH 7.2 for 16 h. The samples were further postfixed in 1% osmium tetroxide for 2 h, dehydrated through an ethanol series, and embedded in a low-viscosity epoxy resin. Ultrathin sections were cut with a diamond knife on a LKB ultramicrotome and mounted on 200-mesh copper grids. They were double stained with 4% uranyl acetate in distilled water for 20 min and Reynold's lead citrate (Reynold, 1963) for 6 min, examined and photographed with a Hitachi H600 TEM. The range and mean of the sizes of phytoplasma bodies were determined by measuring 150 individual bodies in cells of ultrathin sections of TEM photoprints. Only those phytoplasma bodies well in focus were selected for measurement.

Results and discussion

Disease incidence at Olds and Lac La Biche

Disease incidence (DI) varied among locations and plant ages (Table 1). In the first week of July, 1994, 3.4% of the one-year-old plants placed in the field in June at Olds (Field 1) started to show symptoms of leaf reddening and yellowing. DI had increased to 10.8% by September, 1995. Three-year-old plants had a higher percentage of infection compared to two-year-old plants at both locations. Less than 1% of the affected plants examined had phylloid symptoms. Since *G. tataricum* requires 2-3 years to establish, there is a significant risk of plants being exposed to phytoplasma infection.

Isolation of bacteria and fungi from diseased plants

Outgrowths of fungi or bacterial colonies were not apparent around leaf sections from diseased plants. The lack of such colonies clearly indicated that the yellows symptoms on German statice were caused by another source of infection.

Observation of phytoplasma bodies in infected plant cells

Examination of ultrathin sections of a leaf vein from an affected leaf and a phylloid flower pedicel revealed a large number of phytoplasma bodies in the sieve tube cells (Figs. 5 and 6). These bodies ranged in size from 0.37-0.82 x 0.32-0.53 μm (mean = 0.49 x 0.42 μm). They were oval, elliptical or spherical, lacked cell walls, and were surrounded by single membranes. Comparisons of our results on phytoplasma bodies in statice tissue cannot be made with those in the report by Marwitz *et al.* (1986) in Germany due to lack of information in that paper. To the best of our knowledge, this is the first report of a phytoplasma disease affecting German statice in Canada.

In this study, we found that many chloroplasts in the floral shoots of diseased plants showed varying degrees of swelling and disappearance of the chloroplast membrane (Fig. 7). Degeneration of chloroplasts caused by phytoplasma infection was reported in *Nicotiana rustica* L. (Ghosh *et al.* 1988) and *Trifolium repens* L. (Lombardo *et al.* 1970). Ghosh *et al.* (1988) reported that phytoplasmas that grew inside chloroplasts of *N. rustica* were subsequently released into the sieve elements. Our studies did not find phytoplasma bodies in the chloroplasts in the thin sections of diseased tissue. It is believed that the alteration of chloroplasts contribute to the yellowing and chlorosis of diseased plants.

Symptomatology

Leaves of the phytoplasma-infected plants were more lanceolate and twisted than leaves on healthy plants. Reddening usually appeared along leaf margins and tips on the lower leaves (Fig. 2). Yellowing and vein clearing were other commonly seen foliar symptoms. These symptoms were also observed on annual statice (*Limonium sinuatum* (L.) Mill.) plants infected with phytoplasmas (Baker *et al.* 1983; Chang *et al.* 1994). Leaf reddening can be caused by low temperatures, which stimulate high levels of anthocyanin production (Engelhard, 1985). Nevertheless, not all statice plants exposed to low temperatures developed reddening and yellowing on leaves. We observed that diseased plants with leaf reddening were sometimes accompanied by either witches' broom or phyllody symptoms. Other pathogens, such as cucumber mosaic virus (Hein *et al.* 1976) and tomato bushy stunt virus (Novak *et al.* 1980), also have been reported to cause leaf reddening in annual and German statice. However, these viruses often induce leaf mosaic symptoms and never cause phylloid flowers. No virus particles were found in the thin sections of the diseased plants examined in this study. Field observations revealed that statice plants with mild symptoms of yellows disease may still produce some marketable flowers.

Infected plants often failed to produce floral stems or formed pale green spikes (Fig. 4). Flowers on diseased plants were much smaller than those on healthy plants. Bunching, witches' broom or phyllody symptoms appeared in the polytelic synflorescence (an open or indeterminate type inflorescence) (Fig. 3). This was caused by the formation of elongated, upright floral stems from the floral organs. Flowering parts often became so heavy with overgrowth that they fell to the ground (Fig. 3). McCoy (1979) discussed the formation of witches' brooms, which also may be produced through the proliferation of lateral branches. Typical symptoms of witches' broom have been reported on annual statice in Alberta (Chang *et al.* 1994).

Phytoplasmas also can cause phloem lesions in the root system of forest trees (Mclean 1944; Eden-Green 1976; Dyer and Sinclair 1991). Although we did not specifically examine diseased statice plants for this symptom, we found that infected plants were often subject to winter kill and fungal infections, such as *Sclerotinia sclerotiorum*.

The statice plantings surveyed in 1994 and 1995 in Alberta were surrounded by grasses and other forage crops that harbored leafhoppers, which moved freely into the statice. The forages may have served as symptomless carriers and favored the spread of the phytoplasma. Mowing the crops surrounding yellows-affected statice may help to reduce disease incidence. Leafhoppers, which are commonly found in crops affected by yellows diseases, have been reported to transmit MLO pathogens (Grylls, 1979; Tsai, 1979; Purcell, 1985). In Alberta, these insects may survive through the winter as eggs and appear as early as May. They also may migrate from Kansas and Nebraska via South Dakota and North Dakota, reaching Manitoba in May, and continue moving westward into Alberta. If warranted, insecticides could be applied to statice plantings in late May to July to control the leafhopper vectors and spread of the yellows disease. The incubation period of phytoplasmas on *G. tataricum* is unknown.

Phytoplasma diseases are important and widespread on many crops in Alberta, including vegetables and ornamentals (Howard *et al.* 1994). Recently, the disease has been reported on winged everlasting (*Ammobium alatum* R. Br.), pak-choi (*Brassica chinensis* L.), dame's violet (*Hesperis matronalis* L.), and annual statice as new hosts in the province (Chang *et al.* 1992; 1994; 1995; 1996). Chiykowski (1973) also concluded that a MLO disease complex composed of several aster yellow strains and other MLO-associated agents exists in North America. Further research is needed to determine the category of the phytoplasma on German statice. Despite the low disease severity in some areas, the economic impact of this disease on the commercial production of this plant could become significant in Alberta.

Acknowledgements

We thank R. Harris and A. Oatway at the Alberta Environmental Centre for their technical assistance.

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Table 1. The occurrence of a yellows disease in German statice (*Goniolimon tataricum*) plantings at two locations in Alberta in 1995.

Location	Plant age (years)	No. plants examined	Disease incidence (%)
Lac La Biche	2	361	13.0
	3	177	23.7
Olds Field 1	2	638	10.8
	3	145	43.4
Field 2	2	1470	21.4

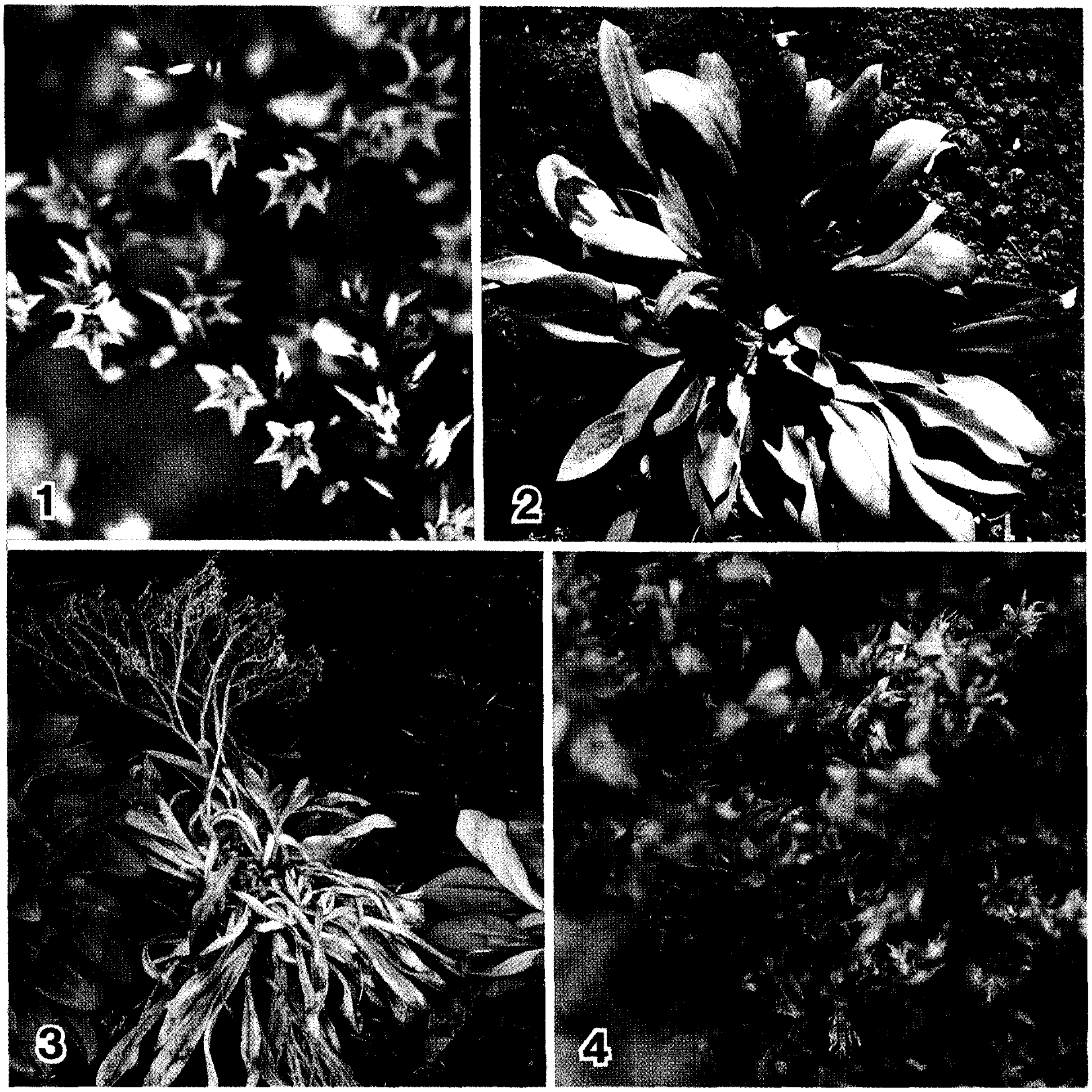

Fig. 1. Healty statice flowers with five-lobed, white calyxes.

Fig. 2. A yellows-affected statice plant showing leaf reddening and yellowing.

Fig. 3. A stunted, yellows-affected statice plant showing chlorosis and reddening of leaves and witches' broom of the inflorescence.

Fig. 4. A closeup of phylloid flowers on a statice plant infected with phytoplasmas.

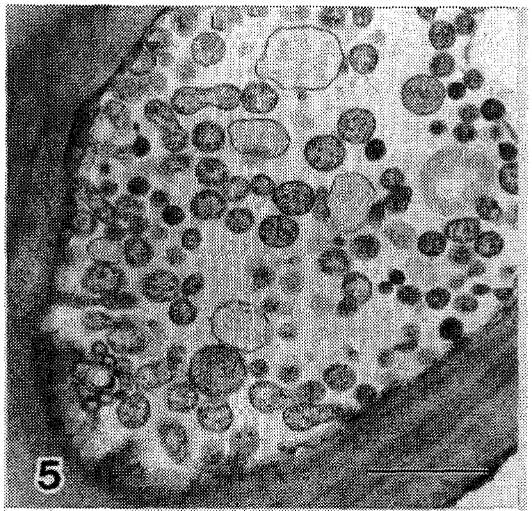

Fig. 5. A thin section of a sieve element from the midrib of a yellow-affected statice plant showing a large number of phytoplasmas. Note the wide variation in sizes. (bar = 1 µm).

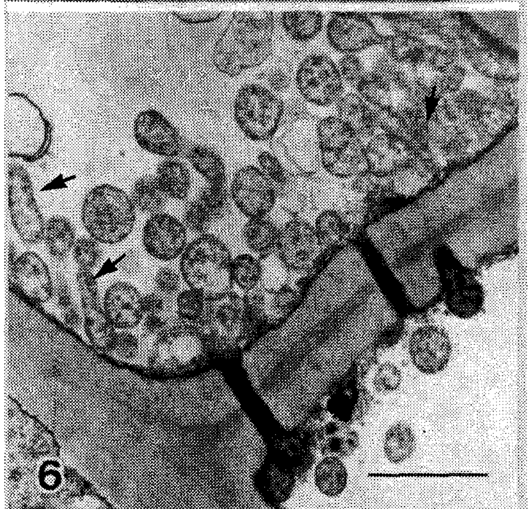

Fig. 6. Phytoplasma bodies in a section through two sieve elements separated by a plate. Most of the bodies are oval to spherical, but a few are club-shaped. (arrow) (bar = 1 µm).

Fig. 7. Swollen and degenerated chloroplasts (arrow) with starch granules in the cell of a young floral shoot of statice infected with phytoplasmas. Note apparently normal chloroplasts in adjacent healthy cells (bar = 5 µm).