Neth. J. Pl. Path. 75 (1969) 123–128

A new disease in tulip caused by Corynebacterium oortii nov. spec.

G. J. SAALTINK and H. P. MAAS GEESTERANUS

Laboratorium voor Bloembollenonderzoek (Flower Bulb Research Centre), Lisse
Instituut voor Planteziektenkundig Onderzoek (Institute of Phytopathological Research), Wageningen

Accepted 20 July, 1968

Abstract

A new bacterial disease in tulip is described. The symptoms consist of: yellow spots on the bulb and silvery streaks and spots or roughened areas on the leaves. Some of the leaf symptoms have been observed in the field for many years, which indicates that the disease has been present for a long time but has never been disastrous. The occurrence seems to be strongly influenced by climatic conditions. Some characters of the pathogen are compared with those of other species of *Corynebacterium*.

Introduction

In 1964 tulip bulbs in storage rooms were occasionally found to show yellow lesions on the first white bulb scales under the brown skin. The bacteria isolated from these spots could not be identified. The next autumn, a greater number of bulbs with similar symptoms were found. It was not until the 1966/67 season that the bacterium was shown to be a pathogen and the symptoms occurring in storage, in the greenhouse, and in the field were determined to be caused by this organism.

Description of the symptoms

The most conspicuous symptom on the bulb is a yellowing of the first white bulb scale (see Plate 1, facing p. 128) which becomes visible when the brown skin is partly cracked or removed. Close inspection shows that this symptom is preceded by the development of patches composed of many small white spots. These patches turn yellow and the tissue becomes raised, often with rupturing of the surface of the swollen area. On a transversely cut surface of a diseased bulb, the tissue of the infected scales shows yellow xylem bundles. Severely diseased bulbs do not sprout after planting but a certain percentage of moderately diseased ones develop into diseased plants. These plants are stunted, show a few silvery streaks along the leaf veins (Plate 1), and wither before flowering. The xylem in the stem of such plants is bright yellow. Microscopical examination of sections of discoloured stem tissue shows numerous bacteria in the xylem vessels.

Plants with silvery streaks were first noticed in greenhouses, mostly in the cultivar 'Paul Richter', and thereafter also in other cultivars in the field. Other symptoms in the field were observed during and after flowering. On the upper surface of the leaf silvery

spots with a diameter of about 5 mm appeared. The epidermis of these spots (Plate 1) cracked very easily when touched and the underlying parenchyma had a disorganized appearance. The leaves of some other plants showed no silvery areas, but they had a roughened appearance caused by a heavy cracking of the upper and lower epidermis. Such plants almost always showed a yellowish discolouration of the central part of the stem that could be followed into the first cell layers of the young growing bulb near the attachment to the stem.
Spread of the disease was considerable in the 1966/67 season, but symptoms were seen only rarely in 1967/68. The roughened appearance of the leaves has been seen for many years, both in The Netherlands and elsewhere. The disease may have been endemic in The Netherlands for many years without serious consequences.

Isolation and inoculation experiments

Identical bacteria were consistently isolated from the rough areas as well as from the discoloured spots or streaks. These bacteria were mainly Gram-positive. Isolates made on nutrient agar from yellow lesions on stored bulbs, however, were usually Gram-negative, but could be made Gram-positive by transferring them from standard nutrient agar to protein-rich agar slopes (Eugon agar). This unstable Gram-reaction may be related to the growth phase of the bulb and the medium in which the bacteria are cultivated. Gram-positive and Gram-negative isolates used for inoculation both caused the same symptoms.
Spraying of a bacterial suspension on the leaves of the cultivar 'Paul Richter' after gentle abrasion of the tissue with carborundum powder, resulted in innumerable silvery spots. Without artificial damage, the infection spots appeared mainly on the leaf tips (Fig. 1). The re-isolated bacteria were identical to the Gram-positive inoculum in every respect.
Injection of a bacterial suspension into the stem of a plant just showing the flower bud, caused silvery streaks in one or two leaves per plant (Fig. 2). These streaks appeared 3 weeks after inoculation, when the plants were held at 17 °C during this incubation period. It is noteworthy that the bacteria could be re-isolated from the basal part of the silvery streak at least 2 weeks after the appearance of this symptom. At 10 °C, however,

Fig. 1. Leaf spots developed on undamaged leaves sprayed with a bacterial suspension

Fig. 1. Bladvlekken na spuiten van bacteriesuspensie op onbeschadigd blad

Fig. 2. Silvery streaks occurring after injection of bacterial suspension into the stem

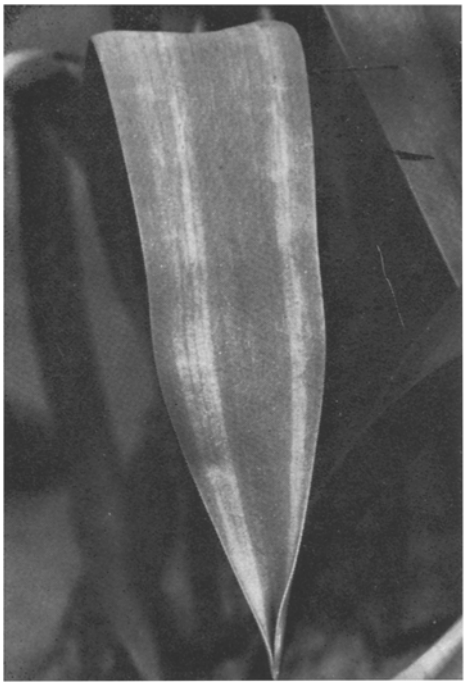

Fig. 2. Zilverkleurige strepen die zich ontwikkelen na injectie van bacteriesuspensie in de stengel

Fig. 3. A spontaneous cracking of leaf tissue after injection of bacteria

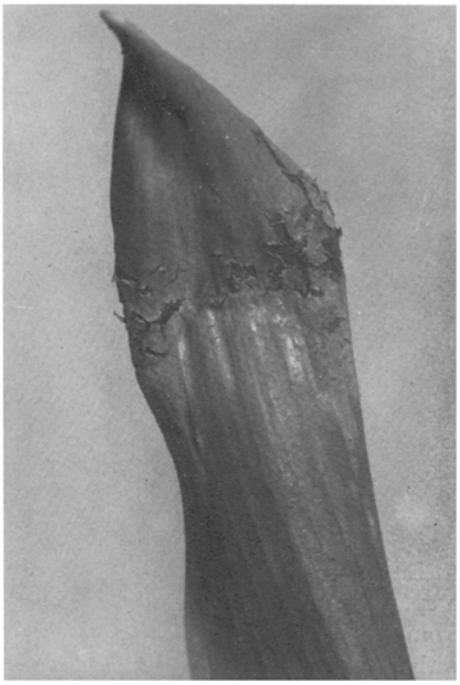

Fig. 3. Spontaan scheuren en opkrullen van de bladepidermis na injectie van bacteriën

no silvery streaks developed. Under these conditions, the leaf tissue cracked spontaneously and became roughened as seen in the field (Fig. 3).
Injection of bulbs with a bacterial suspension also resulted in infection.

Identification of the pathogen

The silvery spots on the leaves closely resembled the symptoms caused by *Corynebacterium betae*, as described by Keyworth and Howell (1961). Cultures received from the National Collection of Plant Pathogenic Bacteria at Harpenden and fromKeyworth were compared with the isolates from our tulips.
Spraying of suspensions of both these cultures of *C. betae* on the leaves of red beet (cv. 'Non plus Ultra') and sugar beet (cv. 'Klein Wanzleben E') after artificial damage by carborundum powder, resulted in typical leaf spots. No symptoms developed on tulip leaves.
An isolate from tulip sprayed on beet and tulip leaves (cv. 'Paul Richter') gave no infection on beet, but a development of typical symptoms on tulip. Injection of bacteria from tulip into the root of beet and the stem of tulip, gave no symptoms on beet but tulip showed the usual silvery streaks.

Table 1. Serological reactions obtained with some *Corynebacterium* species

	Antigens of Corynebacterium						
	oortii	*betae*	*tritici*	*poinsettiae*	*fascians*	*flaccum-faciens*	*michi-ganense*
Antiserum Lisse							
C. oortii	+++	+++	++	–	–	–	–
C. fascians	+	++	++	–	++	–	–
Antiserum Bucharest							
C. betae	+++						
C. poinsettiae	–						
C. flaccumfaciens							
strain 567	–						
strain 706	++++						
strain 712	++++						
C. flaccumfaciens var. *aurantiacum*	–						

Tabel 1. Serologische reacties tussen enige Corynebacterium-soorten

At Lisse, serological reactions obtained with antiserum of the tulip isolate (antiserum prepared with bacteria killed at 60 °C) demonstrated a relation between the isolate from tulip, *C. betae*, and *C. tritici*. Reactions obtained at Bucharest (by Dr I. Lazar) with antisera of different species of *Corynebacterium* (antiserum prepared by injection of live bacteria) showed a relation between the isolate from tulip, *C. betae*, and some strains of *C. flaccumfaciens* (Table 1). The reactions obtained with antigens of other *Corynebacterium spp.* were negative.

Morphological and physiological characters of the isolate from tulip show differences from all known plant pathogenic *Corynebacterium* spp. Although it is closely related to *C. betae* the pathogen isolated from tulips showed the following differences. The flagellae are polar monotrichous; the cells are larger and sometimes club-shaped (Fig. 4); starch is not hydrolysed; growth on nutrient agar is poor; on potato the colour of the culture is dark yellow. Therefore, we consider the isolate from tulip to be a new species.

Discription of the pathogen

Corynebacterium oortii nov. spec.

Named after Dr A. J. P. Oort, Professor of Phytopathology, 1949–1968, at Wageningen, The Netherlands

Pleomorphic rods, size on nutrient agar (average of 50 measurements with the electron microscope) 0.8 (0.5–1.1) × 1.9 (1.3–2.6) micron. (*C. betae* Keyworth et al., under the same conditions: 0.6 (0.4–0.7) × 1.2 (0.7–1.8) micron). On protein-riche media, often club-shaped. Isolated from tulip bulbs during the storage period on nutrient agar: Gram-negative and non-motile; isolated from growing plants: Gram-positive, motile by means of a single polar flagellum, motility stimulated by potato-glucose agar. Gelatin: no liquefaction. Nutrient agar: colonies round, slightly convex, 1 mm in diameter, entire, smooth, and pale yellow. Protein-rich agar (Eugon agar): abundant growth, colonies round, convex, 3 mm in diameter, entire, smooth, viscid, cream coloured. Broth: slightly turbid.

Fig. 4. *Corynebacterium oortii* nov. spec. (× 22,000). On the right: club-shaped cell.

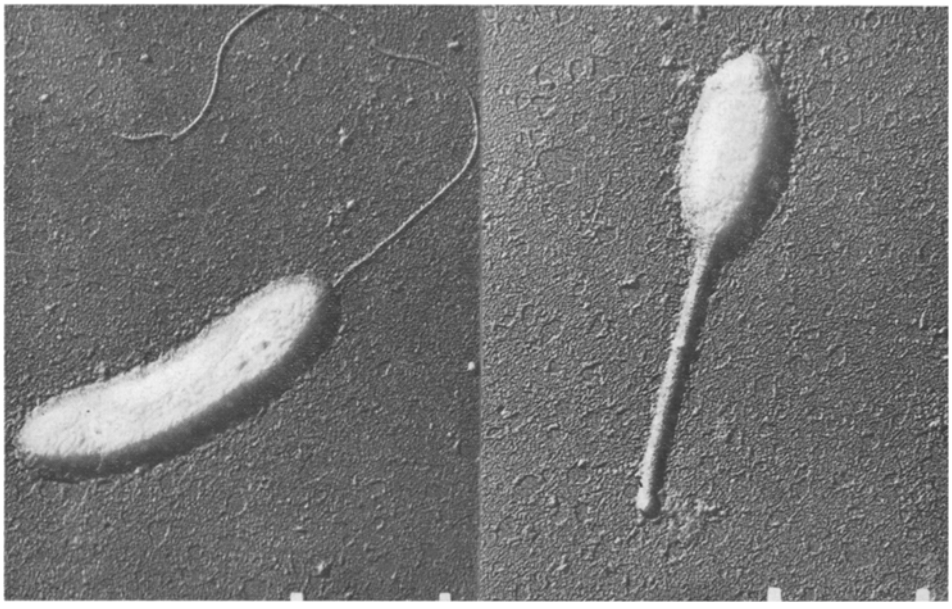

Fig. 4. Corynebacterium oortii nov. spec. (22.000 ×). Rechts: knotsvormige cell.

Litmus milk: no visible change for 1 week, then a soft curd and reduction of litmus. Potato: scanty growth, dark yellow. Indole not produced. Hydrogen sulfide not produced. Ammonia not produced in peptone media. Nitrites produced from nitrates after 10 days. Acid from glucose and sucrose: weak acid from glycerol. No action on lactose maltose, ethanol, esculin. Salts of citric acid not utilized. Urea not hydrolyzed. Acetylmethylcarbinol not produced. Methyl-red test negative. Starch not hydrolyzed. No growth in 4% salt. Positive lipolytic reaction on tributyrin agar. No liquefaction of a sodium polypectate medium. Scanty superficial growth with alkaline reaction, but no liquefaction on carboxymethylcellulose medium. Aerobic. Temperature: minimum 5 °C, optimum 25 °–30 °C, and maximum 37 °C.

Comment: this species is closely related to *Corynebacterium betae* Keyworth, Howell and Dowson (1956). The differences are formed by the flagallae, hydrolyzation of starch, liquefaction of gelatin, growth on nutrient agar, and the difference in colour on potato.
Source: Isolated from yellow spots on tulip bulbs and from leafsymptoms.
Habitat: Pathogenic on tulip.

Cultures of the organism have been deposited in the American Type Culture Collection, Davis, U.S.A. and in the National Collection of Plant Pathogenic Bacteria, Harpenden, England.

Acknowledgments

We are indebted to Dr W. G. Keyworth, Warwick, England and to Dr I. Lazar, Bucharest, Rumania for helpful cooperation, and to W. Kamerman for technical assistance. The colour plate, painted by Mr A. Noordijk, has generously been provided by the Plant Protection Service, Wageningen.

Samenvatting

Een nieuwe tulpeziekte, veroorzaakt door Corynebacterium oortii nov. spec.

In 1967 werd vastgesteld dat in tulp een bacterieziekte voorkomt, waarvan in 1964 de eerste duidelijke symptomen op de bol werden waargenomen. De symptomen in het blad zijn al veel langer bekend. De symptomen op de eerste witte rok van de bol kunnen tijdens de bewaring worden waargenomen nadat de bruine huid is gescheurd. Eerst ontstaan plekken die zijn samengesteld uit witte puntjes ter grootte van een speldeknop. Deze plekken worden geel, zwellen op en scheuren tenslotte vaak met kleine barstjes. Op grond van dit symptoom is de naam "geelpok" aan deze ziekte gegeven.
In een verder stadium van het onderzoek werden bovengrondse symptomen bekend. In de kas en op het veld werden in ontwikkeling geremde planten gevonden, die over de gehele lengte van het blad één of enkele zilverkleurige smalle strepen toonden. De bollen van dergelijke planten toonden altijd de beschreven gele pokken. Op het veld werden bovendien later nog andere bovengrondse symptomen gevonden. Ten eerste zilverkleurige plekken van circa 5 mm diameter op het blad. Ten tweede een scheuren en opkrullen van de epidermis vooral aan de bladpunt. Dit laatste symptoom is al vele jaren bekend onder de naam "helsvuur"; het werd tot nu toe aan een fysiologische oorzaak (kou) toegeschreven. Opmerkelijk is dat van aangetaste bladeren met de genoemde verkleuring reeds bij een lichte aanraking de epidermis scheurt.
Uit alle beschreven symptomen werden herhaaldelijk identieke bacteriën geïsoleerd. Met deze isolaties werden na kunstmatige infectie de beschreven symptomen verkregen. Hoewel de bacterie door vele eigenschappen vrij nauw verwant is aan *Corynebacterium betae* zijn voldoende argumenten gegeven om hem als een aparte soort te kunnen beschouwen.

References

Keyworth, W. G., Howell, J. S. and Dowson, W. J., 1956. Corynebacterium betae (sp. nov.) the causal organism of silvering disease of red beet. Pl. Path. 5: 88–90.

Keyworth, W. G. and Howell J. S., 1961. Studies on silvering disease of red beet. Ann. appl. Biol. 49: 173–194.

Plate 1. A: On the bulb surface patches appear consisting of many white spots, which later extend as yellow patches. A transversely cut diseased bulb shows yellow xylem bundles.
B: Diseased bulbs develop into plants with silvery streaks along the leaf veins.
C: In the field leaves were found with silvery spots on the upper surface. Some leaves looked rough by cracking of the upper and lower epidermis (D). The heavily damaged plants almost always had a yellowish discoloration of the stem (E).

Plaat 1. A: Op de buitenste witte bolrok ontstaan vlekken bestaande uit kleine witte plekjes, die samenvloeien en veranderen in gele vlekken. Een dwarsdoorgesneden, aangetaste bol vertoont geelgekleurde houtvaten.
B: Uit zieke bollen groeien planten met zilverachtige strepen langs de bladnerven.
C: Op het veld werden planten gevonden waarvan de bladeren zilverkleurige vlekken aan de bovenzijde hadden; bij sommige planten was het bladweefsel geheel gescheurd of rafelig na het barsten van de boven- en onderepidermis (D). Dergelijke gehavende planten vertoonden bijna altijd een gele verkleuring van het centrale gedeelte van de stengel (E). Hieruit en uit de bladeren met symptomen worden steeds bacteriën geïsoleerd.

Plate 1