# Occurrence of *Corynebacterium oortii* SAALTINK et MAAS GEESTERANUS on Tulip in Japan

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## Introduction

In 1969, SAALTINK and MAAS GEESTERANUS described from the Netherlands a new bacterial disease of tulip caused by *Corynebacterium oortii* nov. sp.. This disease had been known there by the name of 'geelpok' (yellow spot) of the bulb or 'helsvuur' (hell fire) of the foliage but its cause had remained obscure for many years. The symptoms consisted of yellow spots on the bulbs and silvery spots and streaks on the leaves. Their report immedately drew our attention to a strikingly similar disorder of tulip that has been seen in Japan since around 1960. For the peculiar appearance of affected plants, that has no resemblance to any other established diseases of tulip, it has been called among growers by various names; 'Bakuretsu-byo' (bursting disease) in Niigata prefecture, 'Kusha-kusha' (crumpling) in Toyama prefecture or 'Boroboro' (crumbling) in Fukui prefecture. Its outbreak and severity varied greatly from year to year, field to field and/or cultivar to cultivar and seemed strongly influenced by climatic conditions. Sporadic attempts made by researchers to clarify the cause failed or, at best, resulted in recovery of a few species of irresponsible fungi and bacteria. Thus, it has been eventually regarded as some physiological disorder without any positive evidence. The year 1972 saw an unusual outbreak of this disorder throughout all tulip areas causing considerable losses to the bulb crops. A number of affected tulips were made available to us and these were critically examined in the light of the finding of the Dutch workers. Our study has demonstrated that this disorder is identical with the Dutch disease both in symptomatology and etiology. Results of our identification work is presented here.

## Symptoms

Near the leaf tip, silvery patches of irregular shape and grayish spots with a diameter of 2–3 mm are first observed. The epidermis of these spots is usually cracked at the center to give a cankerous appearance (Plate B, C). The spots may accompany silvery rings or halo patterns. In some plants, silvery streaks are seen on the stem and the flower bud as well. The epidermis of the silvery area often cracks spontaneously or when touched and exposes disorganized underlying parenchyma. In some other plants, a heavy cracking of the upper or lower epidermis occurs without any sign of silvery

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symptoms. Rupturing of the epidermal tissue also appears on the stem mainly close to the flower bud (Plate E). Flower petals may exhibit discolored fleckings and blistering cankers (Plate G). Affected parts of the plants, whether leaf, stem or inflorescense, show various degrees of distortion depending upon the severity of symptoms (Plate F). Plants heavily infected in early growth stage frequently topple down and wither away (Plate A). On the bulbs, yellow blotches can be seen on the first white scale when the coat skin is removed.

In the field, this disease breaks out soon after the emergence of sprouts in early spring. Initial symptoms develop mainly at the tip or middle portion of the first and second sprouting leaves but less often on upper leaves. The symptoms enhance rapidly as the growth of tulip speeds up but they tend to be arrested toward the blooming stage. Though the disease can be observed on most cultivars, it is more common on those cultivars belonging to the breed of Darwin such as William Pitt, Red Pitt, Paul Richter etc. Cottage and Darwin Hybrid groups are also severely affected. Diseased plants appear in patches here and there in a field. There is no apparent relations between the rate of occurrence and the soil conditions or the crop rotation.

## Isolation of the Pathogen

Isolations were tried from both the diseased and the healthy-looking portions of foliage and bulbs. Wakimoto's potato semi-synthetic agar (WAKIMOTO et al., 1968) was strengthened with 5% beef extract and used as a standard medium for making isolations and for maintaining stock cultures. By conventional dilution plating, Gram-positive yellow colonies were consistently recovered in abundance from diseased tissues. Recovery of these colonies from neighboring healthy tissues was negative or, if any, poor and inconsistent. Presence of the Gram-positive bacteria was also demonstrated in the smears from cut sections of diseased stems and leaves.

## Inoculation Experiments

Five isolates (Y–1～Y–5) were selected for pathogenicity tests (Table 1). Inoculum suspensions were prepared from slant cultures (48 hours at 25°C) and adjusted to the

Table 1. Test isolates and their sources

| Isolate | Source of isolation | | Date of isolation |
|---|---|---|---|
| | Cultivar | Locality | |
| Y-1 | Golden Harvest | Arakawa, Niigata | April 30, 1972 |
| Y-2 | William Pitt | Yokogoshi, Niigata | May 11, 1972 |
| Y-3 | Mamassa | Kawahigashi, Fukushima | May 18, 1972 |
| Y-4 | Golden Harvest | Arakawa, Niigata | May 19, 1972 |
| Y-5 | William Pitt | Yokogoshi, Niigata | May 19, 1972 |
| *C. oortii** S152-VD26 | — | the Netherlands | — |

* Type culture of *C. oortii* sent from Dr. G. WESTSTEIJN, Flower Bulb Research Centre, Lisse, the Netherlands

concentration of ca. $10^9$ cells per ml. Using the cultivars William Pitt and Golden Harvest grown in pots in the greenhouse, each inoculum was lightly injected into the ground-level portions of sprouting leaves. All isolates incited water-soaked lesions around the needle punctures in 2 to 3 days. These lesions swiftly turned silvery with swelling epidermis and spontaneously burst open in 7 to 8 days. The bacteria identical with the inoculum source were reisolated from such lesions.

Further inoculations were made by modifying the application of bacterial inoculum. Spraying of the inoculum on young leaves with or without rubbing injuries by carborundum produced innumerable silvery spots on the leaves and eventually induced cracking of leaves and stems and blistering of flower buds (Plate D, G). Growth of infected plants was retarded, the flower buds deformed and the blooming often halted.

Bulbs were inoculated by the following two methods; one by injecting the inoculum into the basal plate and the other by giving a shallow cut into the plate with a smeared scalpel. Inoculated bulbs were soon planted in pots and placed in the greehouse. Both methods resulted in the emergence of typical symptoms on the young sprouting shoots. Patterns of the symptoms and the course of disease development on these plants were exactly similar with those of natural infection in the field.

## The Causal Organism

The following description is based on the study of five isolates (Y–1～Y–5) listed in Table 1. A type culture of *C. oortii* (Isolate S152–VD26) received from Dr. WESTSTEIJN, Flower Bulb Research Centre, Lisse, the Netherlands, was also included for comparison.

### Morphology

The bacterium is a non-spore-forming rod with rounded ends, size 0.8 (0.5–1.3)× 1.6 (0.9–2.6) micron (average of 50 cells from 24 hour-old culture). It is Gram-positive and motile with a single polar flagellum (Plate H).

### Cultural Characters

Beef-extract peptone agar: On the plate culture, colony growth was rather slow; 1.0–2.0 mm in diameter after 3 days at 25°C. The colonies were pale yellow, round, slightly convex, entire, smooth, viscid and glistening. On the slant culture, growth was moderate, filiform, slightly raised. The medium remained unchanged in color. On the stab culture, growth was slight along the puncture but luxuriant on the surface.

Browth: Growth moderate. Slightly turbid without ring and pellicle. A little amount of viscid sediment formed later. The medium did not change in color.

Peptone water: Growth was similar to that in broth.

Potato dextrose agar plate: Growth rather slow. Colonies white with slightly creamy tinge; 2 mm in diameter in 4 days.

Potato semi-synthetic agar (PSA): Good growth. Colonies definite cream-color.

Beef-extract PSA: Luxuriant growth. Colonies viscid and bright yellow; 2–3 mm after 4 days.

Uschinsky's solution: Faintly turbid without ring or sediment.

Cohn's solution: No growth.

Fermi's solution: No growth.

Potato plugs: Growth moderate. Dark yellow colonies.

Litmus milk: No visible change for 5–7 days and then slow peptonization took place following the reduction of litmus and the production of soft curd.

**Physiological Characters**

Oxygen requirement: Aerobic.

Liquefaction of gelatin: No visible change for one week, then liquefaction set in very slowly.

Reduction of nitrate: In peptone water containing 0.1% potassium nitrate, nitrate was not reduced until after 5 days.

Production of indole: Indole formation was not detected with Dowson's method.

Production of hydrogen sulphide: Indication of hydrogen sulphide was not clear on lead acetate paper.

Production of ammonia: In peptone water, ammonia was not detected after 5 days by Nessler's reaction.

Voges-Proskauer reaction: Reaction negative in glucose-phosphate-peptone medium.

Methyl red test: Negative in glucose-phosphate-peptone medium.

Hydrolysis of starch: No hydrolysis in bouillon agar containing 0.2% soluble starch.

Utilization of citrate: Citrate not utilized in Koser's medium.

Hydrolysis of urea: Negative in Christensen's medium.

Production of acid from carbon-compounds: In Barsiekow's medium, acid was produced from glucose, fructose, sucrose, maltose, lactose, glycerol, mannose, xylose, galactose, salicin but not from mannitol, sorbitol and ethanol.

Oxidative fermentation test: Glucose decomposition positive in aerobic condition but negative in anaerobic condition.

Oxydase reaction: Negative.

Catalase reaction: Positive.

Tolerance to sodium chloride: Faint growth in peptone water containing 5% salt.

Temperature relations: Optimum 25–30° C, minimum below 5° C and maximum 37° C. Thermal death point 54° C for 10 min.

**Serological Tests**

Serological properties were examined by cross-agglutination and gel-diffusion tests. Two other species of *Corynebacterium* were included in these tests; *C. michiganense* isolate C1–1–7 (originated from National Institute of Agricultural Sciences, Tokyo) and *C. sepedonicum* isolate 65a (isolated by OBATA in 1970).

Cross-agglutination test: Antisera were prepared against living cells of the isolate Y–1 and the type culture S152–VD26. Cross-agglutinations with living cell antigen have shown that all our isolates are serologically homologous with the Dutch isolate (Table 2). When heated cells (100°C for 30 min.) were used, some of our isolates showed stronger agglutinations. *C. michiganense* was not related to the tulip isolates. Heated antigen of *C. sepedonicum* suggested a distant relation between this species, the tulip isolate Y–1

Table 2. Agglutination reactions obtained between tulip isolates, *C. oortii* and two other species of *Corynebactrium*

| Antigen | | Antiserum | |
|---|---|---|---|
| | | Y-1 | *C. oortii* S152-VD26 |
| Y-1 | L* | ++++ | +++ |
| | H** | ++++ | ++++ |
| Y-2 | L | + | ++++ |
| | H | ++ | ++++ |
| Y-3 | L | + | ++ |
| | H | ++ | +++ |
| Y-4 | L | ++ | +++ |
| | H | +++ | +++ |
| Y-5 | L | + | + |
| | H | +++ | ++++ |
| *C. oortii* S152-VD26 | L | ++++ | ++++ |
| | H | ++++ | ++++ |
| *C. michiganense* Cl-7-7 | L | − | − |
| | H | − | − |
| *C. sepedonicum* 65a | L | − | − |
| | H | ++++ | + |

* Living cell antigen, ** Antigen heated for 30 min. at 100 °C.

and the type culture S152–VD26.

Gel-diffusion tests: Antisera prepared against living cells of the isolate Y–1 and sonicated cells of the type culture S152–VD26 were used. In the reciprocal double-diffusion test, several dominant bands of precipitation were shared in common between our isolates and the type culture. No reactive antigen was found either in *C. michiganense* or in *C. sepedonicum* (Figures 1, 2).

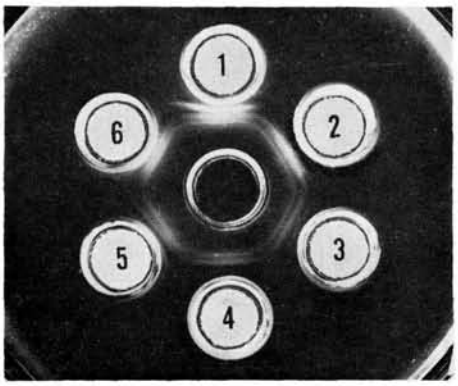


Fig. 1

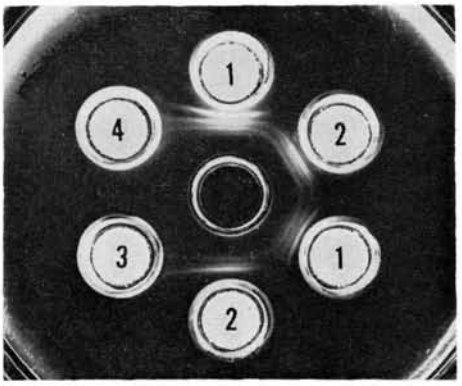


Fig. 2

Fig. 1. Gel-diffusion reactions between *C. oortii* and tulip isolates
Center well: Antiserum against sonicated *C. oortii* S152–VD26
Outer wells: Living antigens of ① *C. oortii* S152–VD26, ② Y–1, ③ Y–2, ④ Y–3, ⑤ Y–4, ⑥ Y–5

Fig. 2. Gel-diffusion reactions between tulip isolates, *C. oortii*, *C. michiganense* and *C. sependonicum*
Center well: Antiserum against living isolate Y–1
Outer wells: Living antigens of ① Y–1, ② *C. cortii* S152–VD26, ③ *C. michiganense*, ④ *C. sepedonicum*

## Discussion

The characters of our tulip isolates coincide with those of *C. oortii* described by SAALTINK and MAAS GEESTERANUS (1969) except in the followings; our isolates liquefied gelatin very slowly, did not reduce nitrate after 5 days, grew slightly in 5% salt and produced acid from maltose and lactose (Table 3). These discrepancies, however, can be attributed to variation in experimental conditions, for the behaviours of the Dutch and our isolates were exactly alike when tested under our conditions. All these isolates were found to be serologically identical but clearly distinct from *C. michiganense* and *C. sepedonicum*. For the specific symptoms induced on tulip, our isolates are also distinct from *C. betae* (silvering disease of red beet) which, according to SAALTINK et al. (1969), resembles *C. oortii* but differs in pathogenicity. On the basis of these findings, it is concluded that our isolates are *C. oortii* SAALTINK et MAAS GEESTERANUS and consequently that the long-pending disorder of tulip in Japan is caused by this organism.

Occurrence of this disease in Toyama, Hyogo and Shimane prefectures was further confirmed in 1973 by specimens sent to the authors for identification. These and other available information indicate that this disease is now widespread throughout our bulb growing regions. In Nagano, Saitama and Ibaraki prefectures in Kanto district, it is said to occur also on forced tulips in the greenhouses and, on occasion, to cause serious damages to the yield of cut flowers. Thus it is of a greater economic importance for the production of bulbs as well as cut flowers.

Outbreak and severity of the disease vary among cultivars. For as yet undetermined reasons, those cultivars suitable for forcing seem to be more often and severely attacked than others (N. FUJII, personal communication). As reported from the Netherlands, there is no doubt that climatic and/or microclimatic conditions greatly influence its outbreak in the field. Soil factors are apparently irresponsible because it is no less common in the paddy soil planted after rice crops or in the newly claimed soil. A major channel of infection therefore is the seed bulbs harboring the causal bacteria. Successive patterns in the emergence of diseased plants in a field further suggest that this disease can be highly epidemic under favorable conditions. The field spread of secondary infection may occur rapidly as well as extensively from a few diseased plants which arise from infected seed bulbs.

SAALTINK et al. (1969) state that the disease may have been endemic in the Netherlands for many years without serious consequences. As to its history in Japan, only two brief records are available from the Plant Protection Service (YAMADA and HASEGAWA, 1960; KOBAYASHI, 1960). These records explicitly show that the occurrence dates back to the years before 1960. The symptoms identical with this disease were seen in Fukui around 1955 (K. NASUDA, personal communication). Farmer's story implicates its emergence in Toyama in the late 1940s. Despite such information, it is suspected of Dutch origin on the following grounds; tulip bulb importation has been steady and nearly one-sided from the Netherlands. Minor infection of imported bulbs has been detected on many occasions in recent years during the post-entry quarantine cultivation.

As a common Japanese name for this disease, the authors suggested the use of

Table 3. Some characters compared between tulip isolates and *C. oortii*

| Characters | Cultures | | *C. oortii* (SAALTINK et al., 1969) |
|---|---|---|---|
| | Y-1 ~ Y-5 | *C. oortii* S152-VD26 | |
| Morphology | Rod | Rod | Rod, pleomorphic |
| Flagellation | Single, polar | Single, polar | Single, polar |
| Size (micron) | 0.8×1.6 | 0.6×1.3 | 0.6×1.7 |
| Gram's staining | + | + | + |
| Oxygen requirement | Aerobic | Aerobic | Aerobic |
| Beef-extract agar | 1.0–2.0mm, pale yellow | 1.0–2.0mm, pale yellow | 1.0mm, pale yellow |
| Potato plug | Growth moderate, dark yellow | Growth moderate, dark yellow | Scanty growth, dark yellow |
| Litmus milk | Reduction positive, soft curd | Reduction positive, soft curd | Reduction positive, soft curd |
| Gelatin lequefaction | + (Very slow) | + (Very slow) | − |
| Nitrate reduction | − (After 5 days) | − (After 5 days) | + (After 10 days) |
| Indole production | − | − | − |
| $H_2S$ production | ± | ± | − |
| Ammonia production | − | − | − |
| V.P. test | − | − | − |
| M.R. test | − | − | − |
| Citrate utilization | − | − | − |
| Urea hydrolysis | − | − | − |
| Starch hydrolysis | − | − | − |
| Oxidase reaction | − | − | |
| Catalase reaction | + | + | |
| O-F test | 0 | 0 | |
| NaCl tolerance | 5% (+) | 5% (+) | 4% (−) |
| Acid from carbon compounds; | | | |
| Glucose | + | + | + |
| Mannose | + | + | |
| Xylose | + | + | |
| Galactose | + | + | |
| Fructose | + | + | |
| Sucrose | + | + | + |
| Maltose | + | + | − |
| Lactose | + (After 72 hr.) | + (After 72 hr.) | − |
| Salicin | + | + | |
| Glycerol | + | + | + (Weak) |
| Mannitol | − | − | |
| Sorbitol | − (After 1 week) | − (After 1 week) | |
| Ethanol | − | − | − |
| Esculin | | | − |
| Thermal death point | 54°C | 54°C | |
| Growth temperature; | | | |
| Minimum | Below 5°C | Below 5°C | 5°C |
| Optimum | 25–30°C | 25–30°C | 25–30°C |
| Maximum | 37°C | 37°C | 37°C |

'Bakuretsu-byo' (bursting disease) in 1972. An alternative new name 'Kaiyobyo' (bacterial canker) proposed later by MUKO et al. (1973) is now considered more appropriate as a standard name for this disease in Japan.

## Summary

Occurrence of *Corynebacterium oortii* SAALTINK et MAAS GEESTERANUS on tulip was described for the first time in Japan. A disorder of tulip called 'Bakuretsubyo' or 'Kusha-kusha' has been known for many years but its cause remained unaccountable. It is now proved identical with the Dutch disease 'geelpok' or 'helsvuur' both in etiology and symptomatology. Detailed descriptions are given on the symptoms, characters of the causal bacterium and mode of outbreak in the field. This disease is now widespread in tulip growing areas throughout the country and is of an increasing concern for the production of bulbs and cut flowers. The name 'Kaiyo-byo' (bacterial canker) is recommended as a standard name for this disease in Japan.

## Acknowledgements

We are indebted to Dr. G. WESTSTEIJN, Plant Pathology Division, Flower Bulb Research Centre, Lisse, the Netherlands for providing a type culture of *C. oortii* and to our colleagues of the Plant Protection Service for their help in collecting specimens.

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## 摘　　要

# チューリップの *Corynebacterium oortii* による新細菌病

小林　敏郎・小畑　琢志
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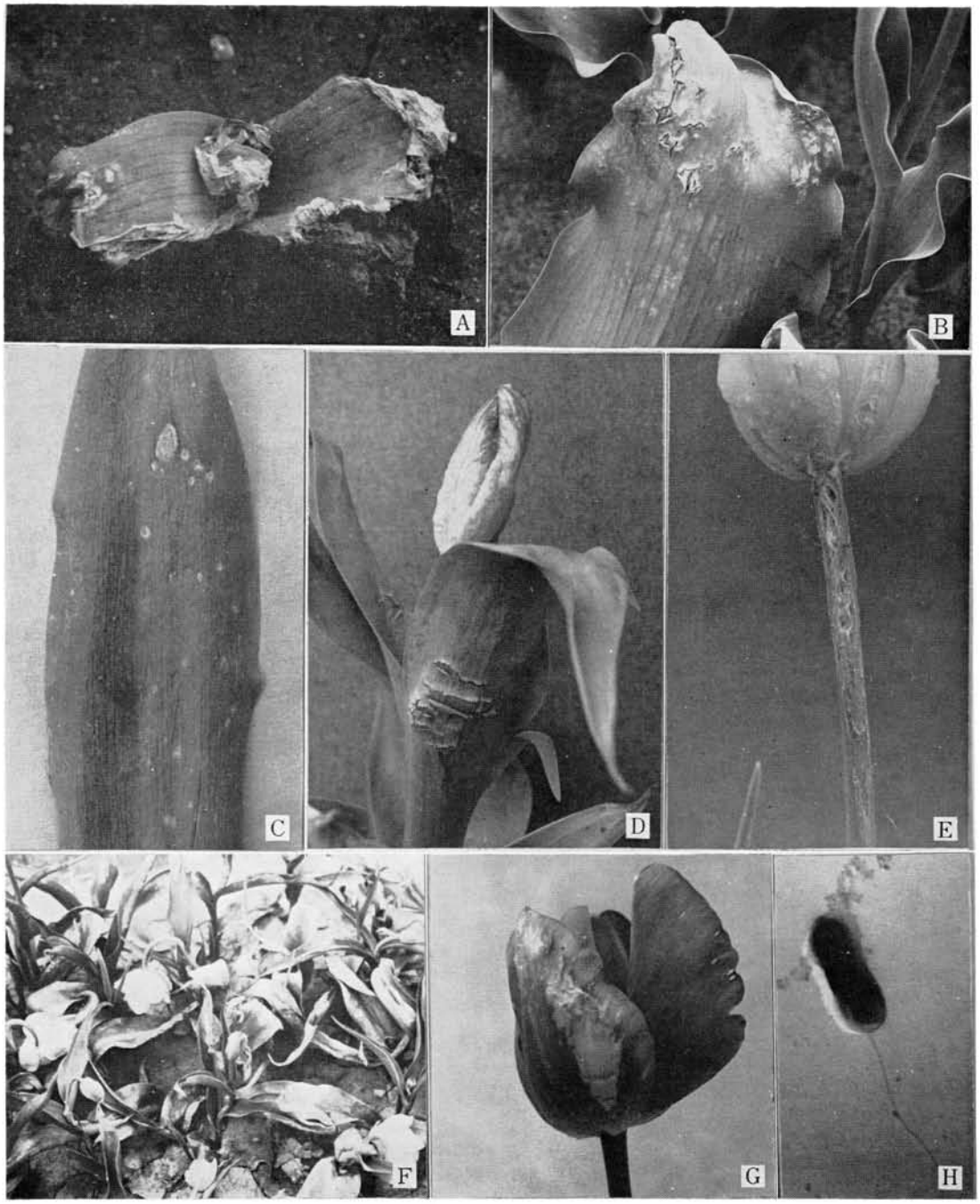
1960年以前からチューリップ球根の産地，新潟県で「ばくれつ（病）」，富山県などで「くしゃくしゃ」，福井県では「ぼろぼろ」と俗に呼ばれる原因不明のチューリップの病害が知られてきた。病徴は葉，花弁，茎および球根に生じ，被害部の表皮は灰白色斑（球根では黄色斑）・亀裂を生じ，かいよう状となり，さらに表皮がはく離し内部柔組織が崩壊して被害部は褐変枯死する（Plate A～G）。発病は崩芽後間もなく認められ，主として第1，2葉の先端部から中央部にかけて進展するが，開花期以降は余り病勢の進展はみられなくなる。品種によって発生程度に差異が認められ，特にダーウィン系の品種に発生が多かった。また栽培圃場の土質や前作の違いによっては発生程度に差は認められず，年ごとに発生程度が異なることから，気象条件の発生への影響が大きいと思われた。

発病株からは常に黄色のグラム陽性細菌が分離され，分離菌を立毛中のチューリップに接種した結果，葉，茎，花弁の表皮に灰白色斑，裂開を生じ，病斑部から接種菌と同一の細菌が再分離された。球根への接種の結果では，生育初期から自然病徴とよく似た病徴を発現した。

病原細菌は大きさ 0.8 (0.5～1.3) ×1.6 (0.9～2.6) μ，桿状で単極毛を有し運動性である（Plate H）。芽胞は認められず，グラム染色陽性である。普通寒天培地上での発育はやや遅く，培養3日目で径 1.0～2.0 mm，淡黄色，全円，表面平滑，やや隆起し，内容均質で湿光を有する。ブイヨン，ペプトン水培養ともわずかに濁り，少量の沈殿を生じた。PSA，PDA 培地上でよく発育し，黄白色，肉汁加用 PSA 培地で発育よく，淡黄色。コーン氏液，フェルミ氏液で発育せず，ウシンスキー氏液でわずかに濁る。ジャガイモ切片上で発育中庸，暗黄色。リトマスミルク培地で牛乳を軟凝固，リトマスをゆっくり還元した。好気性である。ゼラチンを7日目以降ゆっくり液化，硝酸塩を還元せず，インドール，アンモニアを産せず，$H_2S$ の産生ははっきりしなかった。V. P.，M. R. 試験はいずれも陰性。澱粉を加水分解せず，クエン酸塩，尿素は利用しなかった。グルコースなど10種の糖類を利用して酸を産生し，マンニット，ソルビット，エタノールは利用しなかった。5% の食塩中でわずかに発育した。オキシダーゼ活性は陰性，カタラーゼ活性は陽性，グルコースを好気的に分解した。発育適温は 25～30 °C で，5°C 以下および 37°C 以上で発育せず，54°C 10分間で死滅した。分離菌とオランダ産 *Corynebacterium oortii* S 152-VD 26 菌は互いの抗血清によく凝集し，S 152-VD 26 および Y-1 菌抗血清を使用した寒天ゲル内沈降反応で共通沈降帯を形成した。

以上の結果から本病原細菌を，日本では未報告であった *Corynebacterium oortii* SAALTINK et MAAS GEESTERANUS と同定した。

本病の病名は「かいよう病」（向ら，1973）が適当と考えられる。




**A**: A heavily attacked plant with crumpled leaves. Primary natural infection (cultivar 'William Pitt'). **B**: Cracking of silvery lesions near the leaf tip. Natural infection (cultivar 'William Pitt'). **C**: Silvery spots of irregular shape and size, often seen on the top leaf at later growth stage. Natural infection (cultivar 'William Pitt'). **D**: Spontaneous cracking of the lower epidermis caused by spray inoculation. Flower bud is discolored and blistered with many small lesions (cultivar 'William Pitt'). **E**: Severe cracking of flower stalk and petals produced by spray inoculation (cultivar 'Golden Harvest'). **F**: Heavily damaged plants observed in the field at blooming stage (cultivar 'Golden Harvest'). **G**: A malformed flower with a large blister canker and silvery streaks on the petal induced by spray inoculation (cultivar 'William Pitt'). **H**: Electronmicrograph of the causal bacterium.