Neth. J. Pl. Path. 72 (1966): 285–298

CORYNEBACTERIUM FASCIANS AND *BOTRYTIS CINEREA* IN *PELARGONIUM ZONALE*[1]
AN ASPECT FROM THE MANY FACTORS CAUSING THE WILTING OF PELARGONIUM

Corynebacterium fascians *en* Botrytis cinerea *bij* Pelargonium zonale
Een facet uit het complex van factoren, dat wegval van Pelargonium *kan veroorzaken*

H. P. MAAS GEESTERANUS, P. C. KOEK and TH. H. G. B. M. WEGMAN

Institute of Phytopathological Research (I.P.O.), Wageningen
Royal Netherlands Fermentation Industries Ltd., Delft
Horticultural Advisory Service, Arnhem

The investigations on the cause of the "Lent disease" of mother plants as well as cuttings of *Pelargonium zonale* have indicated, that these troubles are due to faulty cultural methodes combined with an infection by *Corynebacterium fascians* and "weakness parasites". The bacteria cause the axillary buds to develop leafy galls on the stem under the soil level and small tumorous growths on the cortex of the stem above, the socalled corky spots. The infected plants may not wither, but galls and spots are more easily infected by pathogens than healthy plants. Frequently *Botrytis cinerea* infects these tissues and causes the black base rot. Spraying the mother plants with streptomycin (Fytostrep 60: 2.5 ml/l) and thiram (0.2%) decreased the base rot, but markedly inhibited the rooting of the cuttings. Moreover, the inocula of both *C. fascians* and *B. cinerea* are present in such a large quantity, that full control is impossible. Reduction of losses may be achieved by improvement of cultural methods and soil disinfection.

INTRODUCTION

History

The village of Lent to the north of Nijmegen has since long been known as a horticultural centre specialized in the cultivation of Pelargonium. During the last year of the second world war this area was evacuated and the entire crop was practically ruined. After the war the district tried to regain its prominence as a horticultural centre and the few remaining nurseries have concentrated on the propagation of Pelargonium to provide new stock for all growers. In doing so, however, the health condition of the plants was neglected and it was later found that a Pandora's box had been opened. Not only was there an increase in virus-infected material, but several fungus diseases occurred as well. The virus infection was controlled by selection, but a base rot remained both on the mother plants and on the cuttings. Eventually this base rot became so general in particular on *Pelargonium zonale* that the phenomenon became known as the "Lent disease".

The growers tried to eradicate the disease by using the then new fungicides, e.g. zineb, thiram and captan, but the treatment did not answer the expectations. Finally research on this problem was started, and attention was paid to fungus control, cultural methods, manuring and water management. Improvements in all these single factors indicate the complexity of the problem. We will illustrate this below.

[1] Accepted for publication 1 October, 1966.

Description of Pelargonium propagation

In the second half of May selected pot plants, the mother plants, are transferred from the garden frames into the open soil. From the end of July until the end of September cuttings are collected from these plants by breaking or cutting. The cuttings are put in the soil to root outside or, at the end of the season, in the frames. After the plants have rooted they are potted and transferred to the garden frames once more to be protected against frost in winter time. During this period watering is nearly stopped. In April of the next year more water is given and the plants start growing again. The cultivation cycle is completed by selecting new mother plants; the remaining plants are sold.

SYMPTOMS AND OCCURRENCE OF THE DISEASE

Symptoms on mother plants

Black or brown soft base rot

Soon after the plants have been transferred into the open, wilting may occur. The larger leaves turn yellow and wither, the top leaves lose their turgescence and become dark green; at the base of the stem a soft rot and often a black discoloration develop. Rotting spreads to about half way up the stem, and then the plant withers. In this stage the root system has totally vanished. If plants showing the first symptoms are pulled up, a witches' broom – or the rotting remnants of it – may be found on the stem below soil level, caused by abnormal swelling of an axillary bud (Fig. 1). When the stem of such a plant is cut longitudinally, the vascular system sometimes shows a brown discoloration.

Soft rot may start at the point of attachment of such a witches' broom, but it also develops on the stem at any place below the soil surface.

Brown dry base rot

Plants may also wilt because their stems become necrotic at the soil surface. In longitudinal sections of these stems a brown discoloration of the vessels is seen, but there is no spreading of the external symptom, except a total drying up of the stem.

The two kinds of wilting mentioned above occur mainly after a period of warm and drying weather; the plants apparently can not recover at night from their shortage of water during the day. In cases in which the roots look healthy, such wilting indicates a blocking of water transport.

Slow wilt

A third kind of wilting may occur while the stem below soil surface appears to be healthy. The roots, however, remain confined to the old pot soil and do not grow into the garden soil. The plant wilts very slowly without any other disease symptom.

Die back

Above the soil soft rot may occur at any place of the stem so that a part or the entire plant dies. This may be rather serious after collecting cuttings under humid weather conditions. This rotting starts from wounds and spreads downward along the stem. At last the stem dries up.

Top rot

At the end of the growing season rotting of the top leaves and buds, which slowly spreads downward, has often been noticed. The young leaves started to rot at the ribs; the older ones wilted. This phenomenon is found only in some cultivars, whereas the other symptoms mentioned may occur in all varieties.

Symptoms on cuttings

Cutting soft rot

Dying of the rooted and not-rooted cuttings is for the greater part caused by a brown or black coloured rotting below the soil surface. This soft rot usually starts where the cutting is cut or broken off, in some cases too at a leaf scar, but it may also develop at any other place of the stem. The difference in discoloration does not seem to be caused by different pathogens; the blackening is probably caused by oxidation of substances in the plant.

Any of the above described symptoms (or a combination of them) is called the "Lent disease", although obviously several causes play a part.

Occurrence on mother plants

The incidence of the disease in the mother plants at different nurseries varies greatly; one grower has little trouble, another suffers a considerable loss. Sometimes the disease occurs only in one or two cultivars in a nursery, while in the neighbouring one these varieties are healthy, but others are diseased. This indicates that minor differences in cultural methods or other factors may be of great influence on the development of the disease.

Nevertheless the disease may be found all year round. During a more or less equable summer we noticed a constant loss of about 4% per fortnight in the mother plants of one of our experimental fields. This percentage increased only after a period of hot and dry weather.

Soft base rot may be found distributed all over the beds, whereas brown dry rot is spreading from one plant only to the surrounding ones.

Occurrence on cuttings

The percentage of surviving cuttings may also vary from nursery to nursery and from cultivar to cultivar. Moreover, we are inclined to think that the cuttings collected in our glasshouse during spring and summer were less susceptible than those collected in autumn. Usually cutting soft rot occurs also scattered throughout the beds, but in a few instances we find only patches of diseased cuttings.

Influence of growing conditions o ι disease incidence

The differences in loss at the various nurseries indicate the influence of slightly varying circumstances of cultivation.

Any inhibition of growth seems to make the plants more susceptible to all kinds of fungi. There is for instance a clear correlation between the incidence of the disease and the local surplus of water due to bad drainage; in other places of this river area near Lent underground gravel layers are common, so that locally the top soil gets desiccated. More attention to watering and shading can decrease the loss of mother plants (DE LEEUW, 1961).

Above we have reported that at some nurseries the roots of the mother plants remain confined to the original pot soil. This phenomenon is common where the composition of this soil differs greatly from that of the soil in the plant beds. In extreme cases desiccation of the pot soil takes place, after which slow wilt (p. 286) occurs.

From the moment of breaking until the wound is closed by callus tissue, the cutting is also less resistant to "weakness parasites". The shorter this period, the less there is chance of infection. A short vulnerable period can be achieved by collecting only actively growing cuttings. Increased losses of cuttings in autumn are probably caused by winter dormancy, induced by the decrease of daylight. Experiments on daylength indicated that, although there were differences between the various cultivars, in general the rooting was promoted by 16 hours of light a day.

COMPARISON OF THE PARASITICAL DISORDERS OF *PELARGONIUM* DESCRIBED IN LITERATURE WITH THE "LENT DISEASE"

It is suggested above that loss is partly caused by circumstances of cultivation; now we want to check which pathogen, described in literature, could play a part in the complex of the "Lent disease".

Insects are not mentioned in literature to cause damage in Pelargonium, although they have been found to spread a pathogen (BUGBEE & ANDERSON, 1963). As a matter of fact wounds caused by insects can be infected by wound parasitic fungi. *Tipula* larvae, for instance, can be the primary cause of cutting soft rot.

The poor growth of the root system might point to an eelworm infection. Inoculations were made by GILLARD & VAN DEN BRANDE (1956), but the development of only slight symptoms did not give rise to further experiments.

Among fungus diseases only a rust (*Puccinia pelargonii-zonalis*) is reported as a real parasite (TRAMIER & MERCIER, 1963; GERLACH, 1965). Well-known fungi such as *Botrytis cinerea*, *Pythium debaryanum*, *Fusarium* sp., *Verticillium albo-atrum*, etc. are incidentally mentioned (NELSON, 1962). Though only little attention is paid to these fungus diseases, they are, however, considered to be potential "weakness parasites" for *P. zonale*. Artificial infection with either *B. cinerea* or *P. debaryanum* caused symptoms each resembling our black soft rot, *Fusarium* sp. caused our brown dry rot (STODDARD, 1957) and *V. albo-atrum* induced wilting (McWHORTER, 1962).

More attention is paid in literature to bacterial diseases (HELLMERS, 1952; KIVILAAN & SCHEFFER, 1958; KNIGHT *et al.*, 1962; LEMATTRE, 1965; MUNNECKE, 1954). *Xanthomonas pelargonii* causes small water-soaked spots which on older leaves become necrotic; on the stem develops a symptom at first rather similar to base rot, but actually it resembles more a dry rot. Infection of the vascular system causes necrosis of this tissue surrounded by a water-soaked area. This disease picture is quite different from the brown discoloration of the vessels mentioned on p. 286.

In Lent this bacterial infection is rare and may be found at the end of the summer. Once we carefully investigated 20 plants with wilting symptoms for the presence of *X. pelargonii*. The bacteria could only be isolated from three of the older leaves of one single plant. All other parts and plants were free from this pathogen. In a few other cases we isolated the white as well as the yellow strains already reported by LEMATTRE (1965).

Since black soft rot can be found abundantly throughout the year and *X. pelargonii* is found here rarely and then only at the end of the season, we need not consider it probable that soft rot is caused by this bacterial species. Therefore *X. pelargonii* needs not be a serious pathogen in the Lent district.

STAPP (1956) reported an infection by *Agrobacterium tumefaciens*. Inoculation with this species can induce tumors on the stem, but we never found natural infection at the nurseries. The illustration in SORAUER's handbook (STAPP, 1956) ascribed to *A. tumefaciens* resembles precisely the witches' broom which can regularly be seen at Lent but which we believe to be caused by *Corynebacterium fascians*. Although it is unlikely that such outgrowths are related to our base rot, a more detailed discussion will be given below.

Although virus diseases consist of leaf spots and leaf distortions, a disorder described by REINERT & HILDEBRANT (1961) is worth mentioning because it resembles our top rot (p. 287). We too were unable to isolate fungi or bacteria soon after the first symptoms of this trouble appeared while there were always virus-like symptoms on the older leaves. In contrast with the base rot, top rot was not affected by fungicide and/or bactericide. We are inclined to believe that our top rot is also due to a virus that occurs in two or three varieties only.

INVESTIGATIONS OH THE CAUSE OF THE DISEASE

Corynebacterium fascians *as a factor causing base rot of mother plants*

Soon after the beginning of the investigations on the cause of base rot it was noticed that nearly all the wilting plants had tumorous outgrowths on the stem below the soil surface (Fig. 2). *Corynebacterium fascians* (Tilford) Dowson was isolated from these excrescences. In adaption to the literature, in the further report we will use the terms: witches' broom, gall, leafy gall, cauliflower or fasciation for the same symptom picture.

To study the correlation between these fasciations and the base rot the following experiment was carried out at a nursery where losses were serious. In the second half of May a great number of potted plants with cauliflowers were put in beds next to beds with an equal number not showing symptoms. In the beds with infected plants, wilting appeared soon, followed by base rot. In the beginning of August 50% of the plants with the cauliflower symptom were dead, whereas in the other beds all plants looked healthy. At the end of September 66% of the diseased plants had died. However, early in September wilting had also started in the group of originally healthy looking plants and by the end of the month the loss of these plants increased to 10%. When all the remaining plants were dug up it was remarkable, that 50% of the group originally with fasciations as well as 50% of the group originally not showing these galls in early spring, had galls in September. Although this experiment clearly demonstrated a correlation between the presence of fasciation on the mother plants and the base rot, the phenomenon, that only half of the originally diseased plants had galls, could not be explained.

The next spring the same experiment was carried out in a more extended and detailed way. About a month before the plants were put in the beds, a large quantity of potted plants was carefully examined and divided into four groups: plants without symptoms, plants with cauliflowers, plants with small cracks in the stems and plants that we had intentionally wounded by breaking off the small cauliflowers. All plants were grown first in pots in frames until the time of planting.

In the second half of May the plants for this experiment were brought in the open, after having been dipped for 15 minutes in a solution of streptomycin (100 ppm Fytostrep 60, Royal Netherlands Fermentation Industries Ltd., Delft), in agrimycin (100 ppm Agri-mycin 100, Pfizer, N.Y.), or in water. The soil of the plant beds had first been disinfected with either Vapam (100 ml/m²) or with Ca-cyanamid (100 g/m²).

During the summer the dead plants were regularly registrated. To avoid wounds as an entrance for additional natural infections, cuttings were not taken during the experiment. From the results the percentages of loss were calculated for the various treatments (Table 1). The experiment included about 1000 plants in three replications at nurseries respectively known as healthy, modera-

TABLE. 1. Influences of soil treatment, plant treatment with antibiotics and symptom expression on the plant on the percentage of base rot of *Pelargonium zonale*.

Invloed van grondbehandeling, behandeling van de plant met antibiotica en de verschillende afwijkingen in het uitgangsmateriaal op het percentage wegval van Pelargonium zonale.

Soil treatment	Plants submerged in:	Plants without cracks and galls	Plants with galls	Plants with cracks	Plants intentionally wounded	Average
Vapam	Streptomycin 100 ppm	22	19	47	53	
	Agrimycin 100 ppm	33	22	39	58	
	Water	20	19	36	42	
	Average/*Gemiddelde*	**25**	**20**	**41**	**51**	34
Calcium-cyanamid 100 g/m² *Kalkstikstof*	Streptomycin 100 ppm	5,5	30	30	50	
	Agrimycin 100 ppm	11	22	30	47	
	Water	2,8	11	33	44,5	
	Average/*Gemiddelde*	**6,4**	**21**	**31**	**47**	26
Untreated *Onbehandeld*	Streptomycin 100 ppm	17	47	56	64	
	Agrimycin 100 ppm	31	33	69	53	
	Water	33	33	61	72	
	Average/*Gemiddelde*	**27**	**38**	**62**	**.63**	48
Average *Gemiddelde*	Streptomycin	15	32	44	56	37
	Agrimycin	25	26	46	53	37,5
	Water	19	21	43	53	34
	Average/*Gemiddelde*	**19,5**	**26**	**45**	**54**	
Grond-behandeling	*Planten gedompeld in:*	*Planten zonder scheuren en gallen*	*Planten met gallen*	*Planten met scheuren*	*Planten verwond*	*Gemiddelde*

tely diseased and diseased. The average losses there were in accordance: 27%, 34% and 46%.

The results in Table 1 show generally that in disinfected soil plants have better chances of surviving than in untreated soil. Moreover, old as well as fresh wounds (cracks and places where fasciations had been broken off) caused a considerable increase of the base rot. In disinfected soil the difference in loss between plants with and without fasciations was negligible. Dipping the plants in a solution of an antibiotic did not have any effect on survival.

When the plants of this experiment were dug up in autumn, again half of them showed cauliflowers. On half of the plants without symptoms in the spring they were found in September. Also only half of the plants which had symptoms in spring, as well as half of those from which the cauliflowers had been cut off intentionally, again showed symptoms in autumn. From these observations we may conclude that during the summer old fasciations may disappear and new be formed. Moreover, infected plants do not invariably show cauliflowers, and as long as we are not sure to work with plants free from *Corynebacterium fascians*, the relation between cauliflowers and the black base rot cannot be clarified.

Occurrence of Corynebacterium fascians *in the soil or on the plant*

We tried to prove that free-living *C. fascians* was present in the soil of the nurseries by sowing dwarf sweet peas (*Lathyrus odoratus* 'Little Sweetheart') in the beds among the Pelargonium mother plants at various times. LACEY (1936) reports namely that infection of germinating sweet peas with *C. fascians* causes small fasciations at the stem base of this test plant. These leafy galls were not found in our seedlings during the spring and the summer. Only a slight infection occurred by the end of September. This may indicate, that the bacteria are not abundant in this soil and probably do not hibernate there. The symptoms of infection on the seedlings in September may be accounted for the fact that the fasciations of Pelargonium fall off and rot away in summer, after that the bacteria invade the soil.

Although infection of the mother plants or the cuttings via the soil of the beds or the frames is doubtful, the occurrence of the fasciations only below the soil surface should point to an influence of soil as an infection source on the host plant. A careful search for the slightest indication of infection of the axillary buds of *P. zonale* above the soil surface remained unsuccessful. On the contrary we did find the cauliflowers in the leaf axils of *P. grandiflorum* (Fig. 3). If the bacteria are apparently present on the stem of *P. grandiflorum* above the soil surface, it would be likely that this is also the case with *P. zonale*.

Fasciations developed only in a small percentage if *P. zonale* was put shallowly in pots with disinfected soil. If later on we put these plants deeply in the pots, either in the same soil or in freshly sterilized soil, we observed leafy galls on all test plants already after one month. This proves that the bacteria in this experiment were present all over the stems of the plants. The environmental conditions in the soil, however, cause the abnormal development of infected axillary buds of *P. zonale* below soil surface, while the infected buds develop normally above.

Artificial inoculation of the leaf axil never produced galls, unless afterwards this part of the plant was put below the soil level. However, rubbing the stem surface above the soil with a mixture of carborundum and a bacterial suspension resulted in the formation of small corky tumors, which we called "corky spots" (Fig. 4). These symptoms were overlooked in the preceding investigations, because they could be found so generally on the plants that one could imagine they belong to the normal appearance of older parts of healthy stems. Repeated artificial inoculation on plants grown from seeds indicated that they were the above-soil symptoms of a stem infection of *Corynebacterium fascians*.

Anatomical investigations showed that in localized areas some cells of the cortex became phellogenic and formed cells with suberized cellwalls in radial rows, phellem (Fig. 5). The whole structure closely resembled a lenticel but differed therewith that only the complementary tissue was suberized, whereas the surrounded cell layers were not. The corky spots do not originate from predestinated initial cells. The fact that the layers of initial cells occur at different distances from the epidermis indicates an abnormality. Some cork formation, not sharply difined areas on the lower part of the stems of old plants, is a normal phenomenon. The symptoms of stem infection, however, are distinct "pimples". Though the young parts of the stem are already infected, the symptoms become clearly visible only in the older portions. From these corky spots bacteria were seldom isolated, probably because they do not survive long in the corky tissue. Better results in isolating the bacteria were obtained by taking the cortex from the younger parts of the stem. The symptoms did not occur on leaves or petioles. The more irregular corky spots, sometimes found there, may be caused by spider mites. When at last we were able to control the bacterial infection, it was also possible to grow plants with absolutely spotless stems.

The pathogenicity of Corynebacterium fascians

To what degree may *C. fascians* cause death in *P. zonale*? For several years we kept plants with galls for demonstration in our glasshouse. In regular inspections we noticed again and again that old fasciations withered and new ones were formed without any harmful effect on the plants. However, when cuttings were dipped in a bacterial suspension before they were put in the soil, about 60 % rotted away and all of the remaining plants had cauliflowers one month later. To keep these diseased plants alive, was not an easy task. Since *C. fascians* is able to liquify slowly a pectate-gel in vitro, the soft rot in these heavily infected cuttings is hereby easily explained.

Assuming that the apparently healthy plants of Table 1 were nevertheless already infected, we isolated a number of plants in a glass-house and sprayed them weekly with either streptomycin (200 ppm), or oxychinolin sulphate (0. 05 %) or copper oxychlorid (0.3 %). For one and a half year we continued this treatment, propagating the crop in the meantime by taking cuttings from these plants. At the end of this period all plants were free from cauliflowers and from corky spots.

Next spring these treated, cured plants were brought in the plant beds and we compared them not only with untreated plants having galls, but also with untreated healthy plants grown from seed to determine if the spraying had any after effect. The soil again was disinfected with either Vapam or Ca-cyanamid or left untreated. In addition one of the replications was sprayed with 300 ppm streptomycin every two weeks (Table 2). Since we did not have equal numbers of plants in the different groups the results are given as a relation: number of dead plants per total number of plants.

TABLE. 2. Comparison of *Corynebacterium fascians* infected plants, cured plants and plants grown from seeds in disinfected soil as to the occurrence of base rot in *Pelargonium zonale*. Results are given as number of dead plants/total number of plants.

Vergelijking van zieke planten ten gevolge van een aantasting door Corynebacterium fascians, *genezen planten en zaadplanten in ontsmette grond op het voorkomen van wegval bij* Pelargonium zonale. *De getallen geven de verhouding aantal weggevallen planten/totaal aantal planten weer.*

	Corynebacterium infected plants	*Corynebacterium* infected plants sprayed with streptomycin	Cured plants	Cured plants sprayed with streptomycin	Seed plants	Seed plants sprayed with streptomycin
Vapam	54/216	32/144	1/24	1/24	0/8	0/8
Ca-cyanamid *Kalkstikstof*	52/216	40/144	2/24	2/24	0/8	0/8
Untreated *Niet ontsmet*	48/216	42/144	0/24	1/24	0/8	0/8
Percentage of stems with corky spots *Percentage stengels met kurkplekken*	97	86	80	56	83	70
Total number of plants with fasciations *Aantal planten met rozetgallen*	130/648	52/432	4/72	11/72	2/24	4/24
	Zieke planten (Corynebacterium)	*Zieke planten* (Corynebacterium) *bespoten met streptomycine*	*Genezen planten*	*Genezen planten bespoten met streptomycine*	*Zaadplanten*	*Zaadplanten bespoten met streptomycine*

At the end of the summer it appeared that the healthy plants grown from seeds were all alive; the treated, cured plants showed little loss, whereas quite a number of the infected ones were lost. Soil disinfection had no effect, but this experiment was done in soil never used for horticulture before and contained evidently too little organisms pathogenic for *P. zonale*. Spraying with antibiotics had hardly any qualitative effect though the amount of corky spots on the stems was somewhat smaller. In a district like Lent where so many Pelargoniums are grown yearly, natural infection is not surprising. It is even doubtful whether control of the bacterial infection will ever be attainable.

The information on the numbers of fasciations is not reliable, in the first place because in this experiment some dormant buds in the soil started to develop and they could not always be distinguished from the cauliflowers, secondly because in the former experiments we have seen that the absence of such galls does not guarantee that the plants are not infected.

The following year a similar experiment was set up, except that soil disinfection was carried out in all beds with vapam. In these beds we put 300 plants collected at random from nurseries, and an equal number of cured plants, which had been sprayed with streptomycin and copper oxychlorid in the laboratory during the winter (Table 3). Unfortunately we did not have a sufficient number of large, cured plants to carry out the experiment at the right time. Therefore this group was completed with potted plants too small for this purpose, of which a number withered soon after transplanting. To correct this disturbing incident we also give in Table 3 the occurrence of the base rot after the period, during which the remaining plants had started to grow. By doing so the disturbing effect of the transplantation on the nursery plants was also adjusted. We did not spray with streptomycin during this experiment, but still there were fewer corky spots on the cured plants than on the nursery plants at the end of the season.

TABLE 3. Comparison of nursery plants and our own cured plants on the occurrence of base rot and corky spots.

Vergelijking van willekeurige praktijkplanten met eigen genezen planten op het optreden van wegval en kurkplekken.

	Nursery plants	Cured plants
Losses throughout the season *Wegval over het gehele seizoen*	33⅓%	14%
Losses after the plants started to grow *Wegval nadat de planten waren aangeslagen*	13%	1.6%
Corky spots clearly present at the end of the experiment *Kurkplekken duidelijk aanwezig*	73%	43%
Only a few corky spots present *Kurkplekken in zeer gering aantal aanwezig*	24%	35%
Corky spots lacking *Kurkplekken ontbreken*	3%	22%
	Praktijkplanten	*Genezen planten*

Correlation between infection by Corynebacterium fascians *and by* Botrytis cinerea *and other fungi*

Table 2 and 3 indicate rather clearly a decrease of base rot if *C. fascians* is absent. Yet we hesitate to state that this species is the only cause of the rotting, because of its low pathogenicity. When making isolations from galls we often obtained also *Botrytis cinerea* Pers. ex Fr., *Pythium debaryanum* Hesse and other "weakness parasites". Moreover, when we followed carefully the rotting process of the fasciations, conidiophores of *B. cinerea* were soon found. The favourable results with soil disinfection in some of the above mentioned experiments also suggest a pathogenic factor occurring in the soil, and this, as we demonstrated with *Lathyrus*, cannot be *C. fascians*. From the many images of rotting fasciations we concluded that these abnormal tissues are more sensitive to infection by soil pathogens than the healthy part of the plants. If these pathogens after having infected the galls, succeed in getting into the stem through the attachment of the gall to the stem, the plant will die. The corky spots in the stem are also potential gates of entrance for pathogens. This dead tissue may rupture at the slightest secondary growth and form the small cracks in the stem listed in Table 1. The effect of these cracks on the occurrence of base rot is also clarified by the data in the table. One may object that the soil disinfection in the first experiment (Table 1) gives only partial control, but we should not overlook the possibility that pathogenic fungi might have entered the witches' brooms and corky spots already before the experiment started.

In general little attention has been paid to the phenomenon that spores of *B. cinerea* can infect some part of the plant and that the mycelium can remain quiescent for a long time before penetrating the surrounding tissues. Therefore we inoculated a number of stems of *P. zonale* with spores of *B. cinerea* early October. Within a few days we could see tiny water-soaked spots on the stems (Fig. 6). After that the parasite remained latent and enlarging of the spots was not seen until the end of February. Then suddenly the spots became larger, the stems turned black, rotting took place and the plants died within a week. A similar phenomenon may occur after leaf infection. The fungus grows through the petiole into the leaf scar and the mycelium may remain dormant there for a long time, while the leaf wilts and falls off. If a cutting with such an infected leaf scar is put into the soil to root, the mycelium penetrates the whole stem and the cutting dies with the soft rot symptom. Sometimes the mycelium does not stay dormant in the leaf scar, but grows through the leaf trace into the stem, until it reaches the vascular system. The infected tissue becomes necrotic, and mycelium growth stops. It looks like an infection by *X. pelargonii*, but differs from it by the absence of the water-soaked area round the necrosis. It is not easy to discover this necrosis at the outside, but if a cutting containes a necrosis of the leaf trace, soft base rot will appear when it is put in the soil to root.

Besides these latent infections *B. cinerea* may also cause quick damage of parts of the plants above the soil, often just after the grower has collected cuttings. Under condition of high humidity the wounds do not dry soon and spores of *B. cinerea* can germinate here and cause a die back of the remaining stem. Moreover it may be possible that spores on the wounds are sucked into the vessels (WILSON, 1964). The mycelium grows downward through the stem and rotting spreads in the same direction.

DISCUSSION AND CONCLUSION

Our six year's research has demonstrated in an indirect way that only part of the complex which causes the "Lent disease" is due to *Corynebacterium fascians*. This bacterial species by itself does little harm. In special circumstances infected plants have even a better chance of survival than healthy ones since the fasciations may form adventitious roots and in periods of drought they obtain moisture from watering, which of course only wets the surface layer, while deeper rooted plants wilt. It was also striking that the best and most quickly rooted cuttings of a sample often showed galls. One can imagine that a bacterial species which causes tumorous growth on the cuttings also will stimulate the callus formation on the wound made by the process of collecting cuttings. The better chance of survival under this special circumstances of both mother plants and cuttings must have been responsible for the wide-spread distribution of infected plants.

When the mother plants are grown under unfavourable conditions in soil contaminated with, for instance, *B. cinerea* or *P. debaryanum*, these parasites infect the abnormal tissue of the fasciations, the cracks caused by rupture of the corky spots or any other wound on the stem below the soil surface.

Subsequently they enter the stem and induce black and brown soft base rot. *Fusarium* sp. might take the same way and causes the brown dry rot. Slow wilt, as indicated, was caused when the composition of the pot soil differed too much from the soil in the beds outside. Die back is mainly an infection by *B. cinerea* and top rot arises probably from infection by a virus.

To indicate the cause of soft rot of the cutting is more difficult. We have reported that a heavy infection of cuttings by *C. fascians* may cause a soft rot. The symptoms are indistinguishable of those appearing after a heavy infection by *B. cinerea*. A slight bacterial infection may stimulate the callus formation on the cut end and this results in a better root formation but here again we cannot distinguish quick rooting healthy cuttings from diseased unless fasciations appear on the leaf scars.

A slight infection by *B. cinerea* seems to retard callus formation on the wounds (NAGY & GLITZ, 1964). We often noticed that rooting occurred only at one side, while on the other a soft rot developed. *B. cinerea* was present there and this phenomenon of partly rooting can be considered as a *Botrytis* infection only.

If the rotting on a cutting stem starts at a leaf scar we are also inclined to think of an infection of *B. cinerea*, but since the galls can be found at these sites, the bacteria must be present there also; thus, the conclusion is doubtful. Moreover, corky spots are often found in a greater number on the stem just above an axil than at other places of the stem. This too indicates that the bacteria may be present in a higher number in the axils. An infection by either *B. cinerea* or *C. fascians* of leaf axils may give therefore the same rotting symptoms.

The combined action of bacteria and fungi on *P. zonale* is also made probable by the generally small losses of *P. grandiflorum*. This difference in loss between the two species is caused by the fact that the more woody stem tissue of this last species is less susceptible to *B. cinerea* and other secondary fungi although the same quantity of galls may be found in both species. On the other hand the bacterial infection is not known in *P. peltatum* and although *B. cinerea* regularly occurs in this plant species, losses of the mother plants of *P. peltatum* are of minor importance in Lent.

Spraying plants with streptomycin 200 ppm every two weeks all year round was successful against bacterial attack, providing the cuttings could root under favourable conditions, i.e. plenty of light and good aeration. Immersion of cuttings in a streptomycin solution for only 15 minutes, however, inhibited the rate of rooting and so decreased the chance of survival. If growers want to use this compound we recommend to stop spraying about a month before collecting cuttings and to start spraying the young plants again after the cuttings have rooted well, to avoid the inhibiting effect of this compound on root formation. The same can be said of some fungicides active against *Botrytis*, because it is known from practical experience that they too inhibit rooting.

Yet either of these chemical compounds cannot give a complete success unless we start Pelargonium cultivation in an isolated place. In the Lent district, where Pelargonium is grown everywhere, we would recommend control by improved cultural measures. Since the soil may be infected, decrease of the disease can be achieved only by disinfection of the soil used for cuttings, potted plants and mother plants. We prefer the use of Ca-cyanamid (1 kg/m^3 pot soil or 100 g/m^2 plant bed, and a three weeks' evaporation period). The biological equilibrium in these intensively used soils is less disturbed by this compound than by vapam and the plants will grow better because of the fertilizing value of its nitrogen. Moreover, special attention must be paid to effective aeration of the crop, cuttings as well as mother plants. If water is needed the leaves should be kept as dry as possible. On rainy days the beds should be covered with glass and they should be also shaded on bright days.

If weather conditions are favourable for infection with *B. cinerea* one should not collect cuttings, because the wounds made on both mother plants as well as cuttings should have to dry quickly. Spraying the mother plants with a fungicide just after collecting cuttings had little effect.

SAMENVATTING

Het onderzoek over de wegval van de moerplanten van *Pelargonium zonale* en het voetrot der stekken heeft aangetoond, dat deze verschijnselen vooral optreden bij een combinatie van minder juiste cultuuromstandigheden en aanwezigheid van een aantal pathogenen. Een dier pathogenen is de bacterie *Corynebacterium fascians*. Deze veroorzaakt op de stengel onder de grond rozetgallen en bovengronds kleine woekeringen van het schorsparenchym, de zgn. kurkplekken. De plaatsen waar deze afwijkingen optreden, worden door allerlei parasitaire schimmels gemakkelijker geïnfecteerd dan het normale planteweefsel. Van daaruit dringen zij de stengel binnen, waardoor de plant afsterft. De meest voorkomende schimmel is *Botrytis cinerea*.

Daar er tussen infectie door *B. cinerea* en het optreden van het voetrot lange tijd kan verlopen, wordt de aanwezigheid van de schimmel in *Pelargonium zonale* te veel als een secundair verschijnsel beschouwd.

Bestrijding van de wegval door bespuiting met streptomycine tegen de bacterie en met thiram tegen de schimmel op de moerplanten werkt in sterke mate remmend op de beworteling der stekken. Vermindering van de wegval kan evenwel worden verkregen door een grondontsmetting toe te passen en te zorgen voor een zeer goede doorluchting van het gewas, moerplanten zowel als stekken, zodat *B. cinerea* bovengronds geen kans krijgt.

REFERENCES

BUGBEE, W. M. & N. A. ANDERSON, – 1963. White fly transmission of Xanthomonas pelargonii and histological examination of leafspots of Pelargonium hortorum. Phytopathology 53: 177–179.

GERLACH, W., – 1965. Eine neue Pilzkrankheit breitet sich in Europa aus: Der Pelargonium Rust (Puccinia pelargonii zonalis Doidge). Nachr. Bl. dt. PflSchutzdienst, Stuttg. 17: 97–100.

GILLARD, A. & J. VAN DEN BRANDE, – 1956. Bijdrage tot de studie der waardplanten van de wortelknobbelaaltjes Meliodogyne hapla Chitwood en M. arenaria Neal. Meded. Landb-Hogesch. OpzoekStns Gent 21: 653–662.

HELLMERS, E., – 1952. Bacterial leafspot of Pelargonium (Xanthomonas pelargonii (Brown) Starr & Burkholder) in Denmark. Trans. Dan. Acad. tech. Sci. 4: 5–40.

KIVILAAN, A. & R. P. SCHEFFER, – 1958. Factors affecting development of bacterial stem rot of Pelargonium. Phytopathology 48: 185–191.

KNIGHT, B. C., A. A. NICHOLS & CHR. W. LEAVER, – 1962. Bacterial leafspot of Pelargonium. Pl. Path. 11: 29–30.

LACEY, M. S., – 1936. Studies on bacteriosis XXII. Ann. appl. Biol. 23: 302–310.

LEEUW, W. P. DE, – 1961. Voetrot bij planten en stekken van Pelargonium. Tijdschr. PlZiekt. 67: 310–311.

LEMATTRE, M., – 1965. La maladie bactérienne du Pelargonium due au Xanthomonas pelargonii (Brown) Starr & Burk. Annls Épiphyt. 16: 5–35.

MUNNECKE, D. E., – 1954. Bacterial stem rot and leaf spot of Pelargonium. Phytopathology 44: 627–632.

NAGY, L. S. & M. GLITZ, – 1964. Injury to vine shoots caused by Botrytis cinerea. Kertész. Szölész. Szölöv. 1: 193–208.

NELSON, L. S., – 1962. Diseases in Pelargonium. Bull. N.Y.St.Flower Growers 201: 2–10.

REINERT, R. A., A. C. HILDEBRANT & G. E. BECK, – 1961. Geranium virus. Florists'Rev. 78 (3323): 17–19.

STAPP, C., – 1956. Bakterielle Krankheiten. In SORAUER, P., Handbuch der Pflanzenkrankheiten, Band 2 Lief. 2, 6e ed., Paul Parey, Berlin und Hamburg.

STODDARD, E. M., – 1957. A Fusarium rot in Geranium and its control. Pl. Dis. Reptr 41: 536.

TRAMIER, R. & S. MERCIER, – 1963. Sur la présence en France d'une rouille du Pelargonium zonale. C. r. hebd. Séanc. Acad. Agric. Fr. 49: 291–295.

WILSON, A. R., – 1964. In: Rep. Scott. Res. hort. Inst. 1963 – 1964: 73.

MCWHORTER, F. P., – 1962. Diverse symptoms in Pelargonium infected with Verticillium. Pl. Dis. Reptr 46: 349–353.

FIG. 1. *Pelargonium zonale* with fasciations on the stem under the soil surface.
Pelargonium zonale *met rozetgallen op de ondergrondse stengeldelen.*

FIG. 2. *Pelargonium grandiflorum* with a witches' broom on the stem which started to rot.
Pelargonium grandiflorum *met een gal op de stengel. De woekering begint te rotten.*

FIG. 3. *Pelargonium grandiflorum* with a cauliflower in a leaf axil.
Pelargonium grandiflorum *met een rozetgal in een bladoksel.*

FIG. 4. Corky spots on the stem of *P. zonale* caused by *Corynebacterium fascians.*
Kurkplekken op de stengel van P. zonale, *veroorzaakt door* Corynebacterium fascians.

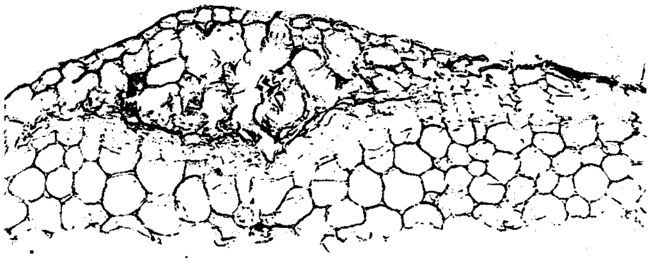

FIG. 5. Cross section from the cortex of a *P. zonale* stem with a corky spot.
Dwarse doorsnede door een kurkplek op een stengel van P. zonale.

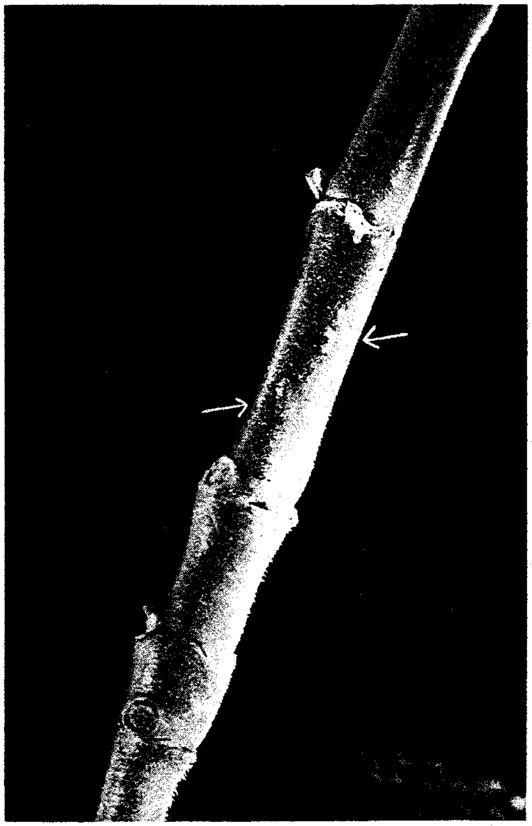

FIG. 6. Symptoms of a latent infection by *Botrytis cinerea* on a stem of *P. zonale*.
Symptomen van een latente infectie door Botrytis cinerea *op een stengel van* P. zonale.