INVESTIGATION OF BULB ROT OF NARCISSUS¹.

PART I. THE NATURE OF THE DISEASE.

By E. J. WELSFORD, F.L.S.

(From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.)

(With Five Text-figures.)

INTRODUCTION.

The date of the first appearance of the disease in this country is somewhat doubtful. Mr J. W. Barr, of Messrs Barr and Sons, states that he first observed it about twelve years ago in some bulbs of *Narcissus Horsfieldii* imported from Holland.

Massee⁽⁹⁾ found *Fusarium bulbigenum* (Cooke and Massee) in Narcissus plants suffering from bulb rot and ascribed the disease to this cause. As, however, no infection experiments were carried out, and as there seems some evidence that *Fusarium bulbigenum* may exist in healthy bulbs, it is clear that the nature of the disease was not settled by Massee's observations. The absence of definite knowledge as to its cause and the increasing ravages of the disease obviously made an investigation very desirable.

Diseased bulbs were obtained in April, 1915, and a preliminary examination showed them to be infested with various animal pests, chief among which were *Rhizoglyphus echinopus*, *Eumerus strigatus*, *Merodon equestris*, and *Tylenchus devastatrix*; certain scales were also found to contain *Fusarium bulbigenum* and bacteria. It was necessary, therefore, to determine which, if any, of the above was the primary cause of the bulb rot.

¹ This investigation was undertaken jointly by the Imperial College of Science and Technology and the Royal Horticultural Society.

INFECTION EXPERIMENTS.

A series of experiments was begun in September, 1915, on bulbs grown in cold frames at the Imperial College of Science and Technology, and in prepared beds at Chelsea Physic Gardens. It was found, however, that more trustworthy results would be obtained if the bulbs could be grown under better conditions, such as those obtaining in the larger nurseries. For this purpose, Messrs Barr and Sons were good enough to provide facilities, and since March, 1916, experiments and observations have been carried out in their fields and drying sheds at Taplow.

Infection with Bacteria.

Infection experiments were carried out with seven strains of bacteria which had been isolated from diseased bulbs and grown on either *Narcissus* or potato-mush agar. Healthy bulbs were planted in September, 1915, and were infected with pure cultures of the several strains in the following month. A piece of the bulb was cut out with a sharp sterilised scalpel and a portion of agar with the bacteria was placed at the bottom of the pit, after which the piece of bulb was replaced. Healthy cut and uncut bulbs were planted as controls. When the plants were examined in the following spring and autumn, they were all found to be quite healthy.

The experiments were repeated during May and June, 1916, but again with negative results.

It was, therefore, concluded that the bacteria which had been isolated from the scales of diseased bulbs were not the cause of the disease.

Infection with Fusarium bulbigenum.

This fungus was isolated from diseased bulbs and grown on sterilised potato-mush agar, it grew vigorously and produced conidia of the *Monosporium* and *Diplocladium* types. A series of experiments was then made; sixty bulbs of the varieties *Narcissus Leedsii*, *Narcissus bicolor* Barri conspicua, *Narcissus incomparabilis* Cynosure, were cut and infected with portions of agar bearing *Fusarium*. The *Fusarium* was used in three stages: (1) rapidly growing non-fruiting mycelium from the edge of a culture; (2) mycelium bearing conidia of the *Monosporium* type, and (3) mycelium with conidia of the *Diplocladium* type.

In every case the bulbs remained perfectly healthy, though the fungus was often found growing between the dead outer scales of the bulb. There was no indication that the fungus had any action on healthy bulbs. The examination of a large number of bulbs leads to the

conclusion that *Fusarium bulbigenum* is very often present on the surface of the dead and dying outer scales of healthy bulbs. It is especially abundant in bulbs which have been injured, but even on rotting scales it seldom penetrates far into the tissues. It is evident, therefore, that the presence of *Fusarium bulbigenum* is not sufficient to account for *Narcissus* rot.

Infection with Black mycelium.

A very dark coloured, almost black mycelium was isolated from diseased bulbs and a series of infection experiments was carried out. The infected bulbs remained perfectly healthy. It was, therefore, obvious that this mycelium did not cause bulb rot, and no attempt was made to obtain conidia or to determine the fungus.

Eumerus strigatus and Merodon equestris.

It has been shown by Fryer(4) that the larvae of these two insects do much harm to *Narcissus* plants, causing a weak, distorted growth, and the loss of a large number of plants. The damage caused by them is, however, quite distinct from the symptoms known as bulb rot.

Infection with Rhizoglyphus echinopus.

These bulb mites are very common on unhealthy bulbs, and are consequently easily obtained for experimental work. The mites were lifted off the diseased bulb with a sable brush and were gently transferred to a healthy bulb, some being put between two injured scales and some between two perfect scales. The bulbs were not planted until the mites were seen to move and were therefore presumably uninjured by the manipulation. These insects undoubtedly do a great deal of harm; they puncture the scales, and gradually cause their destruction, thus weakening the bulb; but in no case was bulb rot obtained by the use of *Rhizoglyphus echinopus*, and it seems, therefore, improbable that the mite causes the disease either by eating the bulb or by carrying bacteria into its tissues.

Infection with Tylenchus devastatrix Kuhn.

During the course of this investigation more than one hundred diseased bulbs have been dissected and in all of them eelworms or their eggs have been found. Small portions of tissue containing live worms were consequently easily obtained and were placed on the cut surface of the scales of healthy bulbs. Fifty bulbs infected in this way were planted in a box of sterilised soil in October and were kept in a cold frame till the following spring. By the middle of February they all

showed the characteristic symptoms of the disease under investigation, namely, precocious growth, an abnormally well-developed root system, blotches on the leaves and, above all, discoloured and rotting bulb scales. No *Fusarium* was observed. Another set of bulbs treated in a similar way, but planted in unsterilised soil, also developed bulb rot. Healthy bulbs, some of which were cut and the rest uninjured, were planted in both sterilised and unsterilised soil as a control; *all* these bulbs remained healthy and showed no symptoms of disease.

When these results are considered in relation to the invariable presence of eelworm in naturally diseased bulbs and to the fact that organisms, such as *Fusarium* and bacteria, which may be found associated with these nematodes, are unable to cause bulb rot, it becomes clear that the eelworm, *Tylenchus devastatrix*, is the sole cause of the disease.

COURSE OF DISEASE.

Healthy bulbs may become infected at any time of the year provided the ground is sufficiently warm for the eelworm to be active. When the tissue in which eelworms are living becomes decayed they forsake it and pass into the ground. In a short time they infect another bulb, entering it either by the bases of the old leaves, which, as is the case with Monocotyledons, are unprotected by a callus, or by young leaves which have been injured and are just pushing up through the soil (Fig. 1). The first noticeable sign of disease in the young leaves is the appearance of pale green areas; these areas become yellow, and, then spreading downwards, produce discoloured "stripes" on the bulb scales. If such an affected portion of leaf is examined, eelworms will be found in the tissue; they lie parallel to the course of the vascular bundles and pass downwards between the cells (Fig. 2). In a few weeks after infection the tissue will contain a large number of eelworms and probably also numerous eggs. It is sometimes difficult to be certain of the presence or absence of eelworms and especially of their eggs, but both can be demonstrated easily by the following method. The material to be examined is fixed in 25 % acetic-alcohol and hand-sections are stained in Ziehl's carbol fuchsin for about an hour, the stain

Fig. 1. Infection at crown on bases of old leaves and on young leaves.

is washed out in alcohol till the plant tissue is almost colourless. By this method the eggs and worms are stained a bright red and are seen as easily in thick as in thin sections.

The infected leaf slowly dies from the tip downwards, and the eelworms, both mature animals and larvae, migrate into the lower portion of the leaf within the bulb. The dead upper portion of the leaf falls on to the ground and if the weather is sufficiently warm the eggs hatch and young larvae are soon liberated in the soil through which they can pass to a healthy bulb. Cold weather makes the eelworms sluggish, or dormant, or even kills them, and consequently checks infection.

Having once entered the bulb, either through the old leaf bases or the young foliage, the eelworms pass slowly downwards to the "plate" leaving the scales discoloured as a whole or in part. If such a diseased

Fig. 2. Eelworms in tissue of bulb scale. $\times 300$.

bulb is cut across transversely the discoloured scale is seen as a brown ring or part of a ring (Fig. 3). If cut longitudinally the diseased scales appear as clearly marked brown stripes (Fig. 4). It is generally the case that bulbs which have a discoloured scale have also an unusually luxuriant root system.

When the eelworms reach the plate they leave the scale down which they have travelled and enter another scale up which they make their way. Thus they are able to reach the green blades again. Blades infected in this way look quite healthy till they suddenly fall over and die. Such a stage is reached in about the month of June when whole masses of leaves may fall within a week. It is obvious that this falling of the foliage is not due to any new outbreak but merely marks a stage in a disease which has been present in the bulbs for many weeks.

As the season advances the diseased bulbs contain an increasing number of eelworms, eggs, and larvae. When such bulbs are removed

to the drying sheds many of the mature worms forsake the scales, no doubt owing to the gradually increasing dryness, and they may be found in bunches among the roots. In this position they are subjected to rapid desiccation and they soon become dormant and fall off, often on to healthy bulbs. If such worms become moistened they revive in a very short time and speedily infect the material around them. Again, if the sheds are warm, the eggs, which have been deposited in the scales, hatch and the larvae attack the tissue in which they are

Fig. 3. Diseased bulb showing ring of brown tissue.

Fig. 4. Diagrammatic drawing of bulb which has been cut in half longitudinally. Second year of attack. Infection spreading up from plate. *a* = crinkly leaf growing at right angles to bulb.

lying. Dry, cool conditions check the spreading of the disease and prevent the occurrence of fresh infection; unfortunately such conditions are difficult to obtain in practice.

When diseased bulbs are planted in the autumn a certain proportion of them die and liberate eelworms which infect their neighbours. Those which survive start growing earlier than healthy bulbs (Fig. 5), and in the spring often produce one or more curved leaves showing crinkled

areas (Fig. 4). Owing to the large number of eelworms in such bulbs it usually happens that the disease spreads quickly, thus separating the scales from the plate; such plates bearing only roots are often found in beds in which the bulbs are very badly attacked. It seems probable that in these cases the disease has started in the previous year.

Fig. 5. Early growth of diseased bulb. August.

GENERAL CONSIDERATIONS.

Eelworm is well known in Europe because of the destruction it causes to various cultivated crops, especially to rye, hyacinth and onion.

Kuhn(5,6) in 1867 and 1868 described a disease in rye and showed that it was due to the action of an eelworm, *Anguillula devastatrix*.

In 1881 Prillieux(10) investigated the "ring" disease of hyacinths and attributed it to an eelworm, *Tylenchus hyacinthi*. Two years later the onion disease was shown by Beyerinck(1) to be due to *Tylenchus Allii*.

Owing to the terrible destruction of hyacinths in Holland the "ring disease," as it is called, has been thoroughly studied in that country, more especially by de Mann⁽⁷⁾ and by Ritzema Bos^(2,3). The latter believes that the slight differences which exist between the eelworms parasitic on rye, hyacinth, onion and numerous other plants are not of specific value but are due to environment. He considers that the eelworm present in these plants should be called *Tylenchus devastatrix*, Kuhn, and he publishes a diagnosis of the species with a detailed description and numerous figures (III. pp. 185, 220). As the characteristics of the eelworm found in Narcissus bulbs in England agree exactly with the diagnosis of *Tylenchus devastatrix* as given by Ritzema Bos, there seems no doubt of the correctness of the identification here put forward.

According to Ritzema Bos (III. pp. 232-234), *Tylenchus devastatrix* attacks a variety of cultivated and wild plants, and though it is capable of passing from one to the other it does not do so easily. He concludes that there are physiological varieties of the species and says: "Il paraîtrait donc qu'on peut admettre comme règle, que le *Tylenchus devastatrix* se fixe dans les plantes dans lesquelles les ancêtres ont vécu depuis plusieurs générations, de préférence à d'autres espèces de plantes; et que, toutes choses égales d'ailleurs, il préfère ordinairement la plante ayant une parenté étroite avec celle dans laquelle vivaient les générations précédentes, à celle qui en est plus éloignée dans le système.

"J'estime qu'on pourra trouver dans ce qui précède la clef de bien des choses, inexplicables jusqu'ici relativement à l'apparition et à la disparition des maladies vermiculaires dans nos cultures.

"Il résulte d'ailleurs de ce qui a été traité jusqu'ici dans ce chapitre, que, si au point de vue *morphologique* il faut considérer comme une espèce unique les *Tylenchus* des jacinthes, ceux de l'oignon, ceux du seigle, du sarrasin, du trèfle, etc., et ceux de la mousse *Hypnum cupressiforme*, il existe néanmoins des différences *physiologiques* entre les différentes *Tylenchus*, selon que leurs ancêtres ont vécu durant un grand nombre de générations, dans telle ou telle plante."

The differences between the eelworms of hyacinths and of onions indicated by Ritzema Bos suggest a comparison with the "biologic forms" described for various parasitic hosts. It is to be noted, however, that Ritzema Bos ascribes to the eelworms merely *preferences* for their original host while the biologic forms are normally confined by physiological differences to their particular host or set of hosts. The physiological differences between the onion and hyacinth "forms" of eelworm,

44 *Investigation of Bulb Rot of Narcissus*

if they exist, are obviously less definite than those which differentiate the biologic forms of the Uredineae and of the Erysiphaceae.

If the eelworm of *Narcissus* follows the behaviour described by Ritzema Bos for that of the hyacinth and of the onion it is clear that while the bulbs are in the ground there is very little fear that it will migrate to neighbouring weeds. However, directly the bulbs are lifted any eelworms remaining in the ground are likely to migrate to suitable weeds¹ which thus would become valuable traps. It is desirable, therefore, that before replanting the ground all weeds should be removed and burnt; weeds should never be allowed to wilt on the soil nor should they be dug in.

A list of the more common British weeds and cultivated plants which have been described as affording shelter to *Tylenchus devastatrix* is given here. It is compiled from papers of Ritzema Bos(2,3); it has no claim to completeness.

Medicago sativa	Hyacinthus romanus
Trifolium pratense	„ orientalis
Polygonum fagopyrum	„ precox
„ convolvulus	Tulipa gesneriana
„ persicaria	Lilium candidum
„ lapathifolium	Fritillaria imperialis
Bellis perennis	Galtonia candicans
Ranunculus acris	Scilla sibirica
Geranium molle	„ campanulata
Sonchus oleraceus	„ cernua
Myosotis stricta	Narcissus Tazetta
Plantago lanceolata	„ Pseudo-narcissus
Poa annua	Muscaria botryoides
Holcus lanatus	„ comosum
Brassica rapa	Humulus lupulus
Anthoxanthum odoratum	Phlox
Centaurea jacea	Chelone glabra
„ cyanus	Solanum tuberosum
Spergula arvensis	Wheat
Allium cepa	Rye
„ proliferum	Oats
„ vineale	Vicia Faba
„ schoenoprasum	

¹ The question of the occurrence of migration and its extent would be well worth investigation.

Ritzema Bos found eelworms in some plants of *Narcissus Tazetta* which he had planted in soil infested with the eelworm of barley, but he states that bulb growers in Holland find that *Narcissus* bulbs do not suffer from bulb rot. He says (III. p. 230) "J'appris aussi par des cultivateurs de bulbes à fleurs de Haarlem, que les tulipes, les lis, la couronne impériale et les narcisses ne sont jamais atteints de la maladie causée par les anguillules (maladie annulaire)."

Thus in 1887 it is clear that the eelworm disease of *Narcissus* was little known in Holland though it was then very prevalent among Hyacinths.

The occurrence of eelworm disease in *Narcissus* is mentioned by Marcinoswski(8) in 1910 but no account is given of it and it seems reasonable to conclude that though it was recognised in Holland in 1910 it was comparatively rare.

Hewitt(4A), working in Ireland, seems to be the only other investigator who has described the occurrence of *Tylenchus devastatrix* in *Narcissus* bulbs. His paper is short and deals mainly with the action on the bulb and organism of various "steeps." In the light of our present knowledge it is clear that he was dealing with "bulb rot"; but his description of the diseased condition is so brief that apart from the nature of the infecting organism the nature of the disease he describes would remain uncertain.

The account given here of the cause and progress of the bulb rot of *Narcissus* shows that certain precautionary measures will check to some extent the ravages of the eelworm. Such measures may briefly be summarised as follows:

- (1) *Narcissus* should not be planted in ground previously infected with *Tylenchus devastatrix*; the danger is especially great if the eelworms have been liberated from a previous crop of *Narcissus*.

- (2) Care should be taken to plant healthy bulbs only; one diseased bulb will soon infect many others.

- (3) When a bulb does not produce foliage at the proper time it should be dug up and burnt before it rots and liberates eelworms in the soil.

- (4) Bulbs with "crinkly" or very curved leaves should be burnt.

- (5) Dying leaves should be gathered and burnt before they can fall on the soil and so possibly infect it.

- (6) Weeds growing in infected soil should be pulled up, put directly into baskets, and burnt,

SUMMARY.

The bulb rot disease, or ring disease, of *Narcissus* is due to the attack of an eelworm, *Tylenchus devastatrix*. These eelworms are of constant occurrence in the diseased bulbs, and the disease can be produced by infecting healthy plants with the eelworm.

A description is given of the symptoms and course of the disease as it appears in nature, and certain precautionary measures are suggested.

I have great pleasure in expressing my thanks to Mr J. W. Barr (Messrs Barr and Sons), Mr Hales (Chelsea Physic Gardens), Mr Leake (The Floral Farms, Wisbech), Mr Pearson (Messrs J. R. Pearson and Sons), and Mr J. Walker (Walker Bros.) without whose cordial co-operation and generous help the work could not have been carried out. I am also indebted to Mr J. C. Fryer for help and suggestions with regard to treatment.

I should also like to take this opportunity of thanking both Professor V. H. Blackman and Professor J. B. Farmer for their stimulating and helpful criticism.

LIST OF PAPERS QUOTED.

1. BEYERINCK, M. W. "De oorzaak van der kroefziekt van jonge ajuinplanten." *Maandblad der Holl. Maatschappij van Landbouw*, No. 9, 1883.
2. BOS, RITZEMA. "Untersuchungen über *Tylenchus devastatrix*." *Biol. Centralblatt*, 1887-8. Bd. VII, p. 232.
3. — "L'Anguillule de la Tige (*Tylenchus devastatrix*) et les Maladies des Plantes dues à ce Nématode." Haarlem. *Musée Teyler Archives*, Sér. 2, 1887-1892, p. 161.
4. FRYER, J. C. *Narcissus Flies*. Board of Agriculture and Fisheries Leaflet, No. 286, 1914.
- 4A. HEWITT, T. R. Eelworms in Narcissus Bulbs. *Jour. Dept. Agric. and Tech. Instit., Ireland*, XIV, 1914, pp. 345-353.
5. KUHN, J. *Zeit. d. Landwirthschaftlichen Centralvereins der Provinz Sachsen*, 1867, p. 99.
6. — *Ueber die Wurmkrankheit des Roggens und über die Uebereinstimmung*.
7. DE MANN, J. G. *Die freie in der reinen Erde und im Süssenwasser lebenden Nematoden der niederlandischen Fauna*, 1884, p. 142.
8. MARCINOSWSKI, K. "Parasitische und semi-parasitische an Pflanzen lebende Nematoden." *Arbeiten aus der Kaiserlichen Biologischen Anstalt f. Land- und Forstwirtschaft*. Bd. VII, 1910.
9. MASSEE, G. "A disease of Narcissus bulbs." *Kew Bulletin*, 1913, p. 307.
10. PRILLIEUX, E. "La Maladie vermiculaire des Jacinthes," *J. de la Soc. Nat. d'horticulture*, 3^{me}, sér. III, 1881, p. 253.