Neth. J. Plant Path. 70 (1964): 136–141

SOME DATA CONCERNING THE PRESENCE AND BEHAVIOUR OF SPRINGTAILS (COLLEMBOLA) ON GRASS AND WHITE CLOVER[1]

G. KOOISTRA
Institute of Phytopathological Research (I.P.O.), Wageningen[2]

No proof was found that Collembola prefer white clover to grass in sandy soils of leys and grasslands. The same springtail species can be found on both. However, in greenhouses they definitely preferred the white clover. Here, *Folsomia quadrioculata* especially, and during the summer months *Sminthurinus trinotatus*, were found. In winter, *Onychiurus bicampatus* or a related species was also identified. *Folsomia quadrioculata* is able to feed on white clover roots. In doing so, invasion spots are prepared for weakly parasitic soil fungi. Addition of these fungi to the soil, however, did not result in an infestation of the roots followed by death and disappearance of the white clover, for *Folsomia* then preferred to feed on the fungi rather than on the roots.

INTRODUCTION

ENNIK *et al.* (1962) mentioned springtails (Collembola) as the possible parasitic cause of the disappearance of white clover in leys and grasslands on sandy soil. This suggestion was based on information from I.P.O. research workers, that many springtails were found in untreated pot cultures of white clover whereas pot cultures treated with the nematicide DD were found to be free from them. They suggested that the springtails and possibly also other species of the soil fauna do not kill the white clover through eating the roots, but that they create points of entry for weakly parasitic soil fungi (*Fusarium*, *Rhizoctonia*, *Cylindrocarpon*, etc.), which could injure and eventually kill the white clover roots. Among representatives of the soil fauna which it was considered might cause infection sites on the roots were Nematoda (e.g. *Heterodera trifolii* Goffart), Collembola, Oribatidae and Sciaridae larvae.

In order to obtain further knowledge of the part played by springtails in the disappearance of clover, an investigation was made in 1962 and 1963 into their presence and behaviour in grass and white clover.

METHODS

Field and greenhouse investigations into the presence of springtails in grass and white clover

In the neighbourhood of Wageningen regular examinations were made of Collembola in three old leys. These three leys had formerly been used as experimental plots by Ir. G. C. ENNIK, Institute for Biological and Chemical Research on Field Crops and Herbage (I.B.S.), Wageningen. On two of the

[1] Accepted for publication 9 May, 1964.
[2] Present address: Laboratory for Flowerbulb Research, Lisse.

pastures the white clover had fallen back to a small percentage of the total crop; on the third it had maintained a growth of approximately 30%. Samples were taken from the top soil to a depth of 10 cm. The drill used belonged to a micro-Berlese apparatus, copied from a model invented by Mr. C. F. VAN DE BUND, Plant Protection Service (P.D.), Wageningen. In addition, with the help of the classic Berlese apparatus, the number and kinds of springtails present in pot experiments with grass[3] and white clover were determined at regular intervals.

The principle of the two types of apparatus is the same: Collembola are sensitive creatures, which fear both light and the threat of drying up; thus by the application of heat and light above the soil they are made to migrate deeper into the earth and eventually fall via a sieve into a flask containing water or alcohol. In this way, with the temperature not exceeding 35 °C, the number of springtails per soil sample can be established and the species present can readily be identified.

Springtail behaviour experiments in glass cuvettes and pots

When commencing to study the combinations of soil fauna and flora mentioned in the Introduction, the difficulty arose as to which insects and fungi should be used. Fungi of the genera *Verticillium*, *Rhizoctonia*, *Tubercularia*, *Cylindrocarpon* and *Fusarium* were chosen at random. These species were isolated from "diseased" white clover roots and cultivated on sterilised soil-oatmeal mixture. Of the springtails, the species *Folsomia quadrioculata* (Tullberg) was

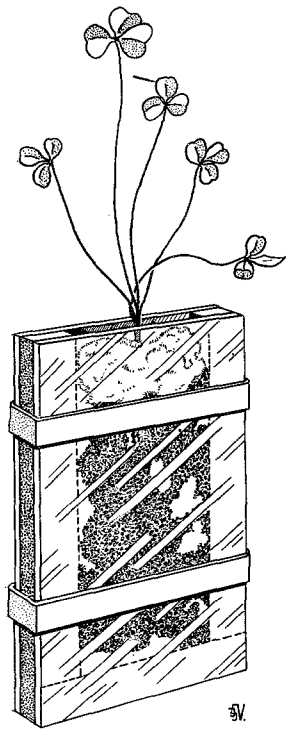

FIG. 1. A glass cuvette into which has been inserted 6 cm³ of sterilised potting soil and one white clover seedling; 50 springtails were later added to the soil and the open end of the cuvette was plugged with cotton-wool.

[3] In pot experiments a monoculture of perennial ryegrass was always used.

chosen because it was easily available. In glass cuvettes measuring $10 \times 4 \times 0.3$ cm, one side detachable, and containing 6 cm^3 sterilised potting soil, one white clover and one perennial rye grass seedling, both 3-4 weeks old, were planted; in some cuvettes only one plant of either kind was planted. Fifty living *Folsomia quadrioculata* specimens were then added to the cuvettes and in a number of cases *Fusarium*, *Rhizoctonia* or *Cylindrocarpon* were also introduced. The moisture content of the soil was made optimal, after which the cuvette was closed in the following manner. The detachable lid was replaced in such a way that the open end could be filled with cotton-wool to allow the green shoots to emerge; the roots and *Folsomia* and any possible fungus present stayed in the cuvette (Fig. 1). The soil moisture in the cuvettes was maintained by placing them in a box of damp peat-dust.

In the pot experiments the five fungus cultures were thoroughly mixed with sterilised potting soil and used in pots in which three white clover seedlings, approximately three weeks old, were planted. About one month later, 100–150 specimens of *F. quadrioculata* were introduced into a number of the pots.

RESULTS AND DISCUSSION

Field and greenhouse investigations into the presence of springtails in grass and white clover

Nearly all the springtails in the field were found in the top five centimeters of the soil. In May 1962, 160–170 Collembola per liter of soil were found in the first ley containing a small percentage of white clover. In July and August the numbers had sunk to 30–50 and in September and October only 5–25 per liter of soil were found. There was no difference between the numbers under white clover and under grass. The species *Onychiurus bicampatus* Gisin, *Isotoma viridis* Bourlet, *Isotoma violacea* Tullberg and *Hypogastrura* sp. were the most prevalent; specimens of the genera *Anurida*, *Folsomia* and *Friesia* were also found, and sometimes specimens of *Odontella armata* Axelson; during the summer months *Sminthurinus* species were to be seen. The identification of *Onychiurus bicampatus* Gisin caused some difficulty. According to GISIN (1960) this species is to be found only on the Danish Islands; it is thus possible that the above-mentioned *Onychiurus* may be a new species closely related to *O. bicampatus*.

On the second ley, containing more, but still very little white clover, springtails were found in greater numbers. In July, under white clover, 200–300 insects per liter of soil were found, whereas under grass these were 100–200. In August the numbers had decreased to 24 per liter of soil under white clover and to 82 under grass. In September, an increase was noted, there being 125 per liter of soil under grass and 57 under white clover. In October and November the numbers per liter of soil under grass and white clover were equal, now reduced to 30. At this time *O. bicampatus* and species of the genus *Anurida* were found to be the most abundant.

On the third ley, with 30% white clover, at least 200 springtails per liter of soil were found in September, but in October and November there were less than 30, species of *Onychiurus* and *Anurida* once again being dominant.

In December 1962, just before the frost period, samples were taken again from these three fields and it was found that they then contained more springtails

than in October and November; especially many *O. bicampatus* specimens were counted. It is suspected that this species reaches its highest breeding point in the winter. In contrast to this, the species *Sminthurinus trinotatus* Axelson was found in its greatest numbers during the summer months. A clear picture of damage caused by the various springtails to white clover in the field was not obtained.

In the grass and white clover pot experiments in the greenhouse the Collembola rapidly increased in numbers. After a period of 4–5 months 160 specimens (an average of 11 observations) were found in pots containing grass; in the pots containing white clover, however, there were 450 per pot (again an average of 11 observations). As these experiments were begun with steam sterilised soil, the insects came into them by natural infection.

During the summer and autumn the species *Folsomia quadrioculata* was especially prominent; also a few specimens of *Sminthurinus trinotatus* were always to be found. In pot experiments in the winter, besides *F. quadrioculata*, many specimens of *O. bicampatus* were found, which again points to the suspicion already expressed, that this species reaches its maximum breeding point in the winter. The results of these experiments in the greenhouse differed from the observations in the field only in the numbers of springtails found beneath the white clover and grass, but not in the species living on them.

Springtail behaviour experiments in glass cuvettes and pots

Ten glass cuvettes with white clover and grass seedlings were placed in a box containing moist peat-dust. Into five of these *F. quadrioculata* was introduced, the other five remaining without springtails. It was soon evident that both the white clover and the grass were developing satisfactorily under these conditions. The experiment was kept up for two months and the growth trimmed three times. The numbers of *F. quadrioculata* also expanded; before long they were to be found in all the cuvettes, including in those that were left without them, as the cotton-wool proved to be an insufficient seal. Within a week from the beginning of the experiment eggs and young larvae were found. The springtails preferred to feed on dead and dying white clover roots; if there were not sufficient of these, the adult *F. quadrioculata* would eat the living roots, especially if these were old ones. In this way the whole cortex right down to the vascular bundles could be eaten. Damage was also noted at the collar of the plant. Against the glass of the cuvette excrement could be seen (Fig. 2). In four of the cuvettes *Fusarium* or *Rhizoctonia* had been introduced. At the places where these fungi were inoculated many springtails were noted; it seemed as though these insects preferred the fungi to the roots. Through this preference they kept the badly eaten and dying roots free from fungi (and presumably bacteria). The grass roots were not eaten as long as they were in a vigorous condition (Fig. 2).

Because the cotton-wool plugs were not a sufficient seal, *F. quadrioculata* specimens escaped and invaded even the peat-dust. At the end of the experiment with the ten cuvettes and the peat-dust approximately 5000 *F. quadrioculata* were caught in the Berlese apparatus. This result showed that there had been a 20-fold multiplication, since the experiment began with 250 springtails. Two thousand living specimens were put into a pot containing three white clover seedlings. In the beginning the white clover did not suffer at all from this great number of springtails. After the first crop analysis, that is five weeks after the insertion of the springtails, it became evident that the white clover, after being

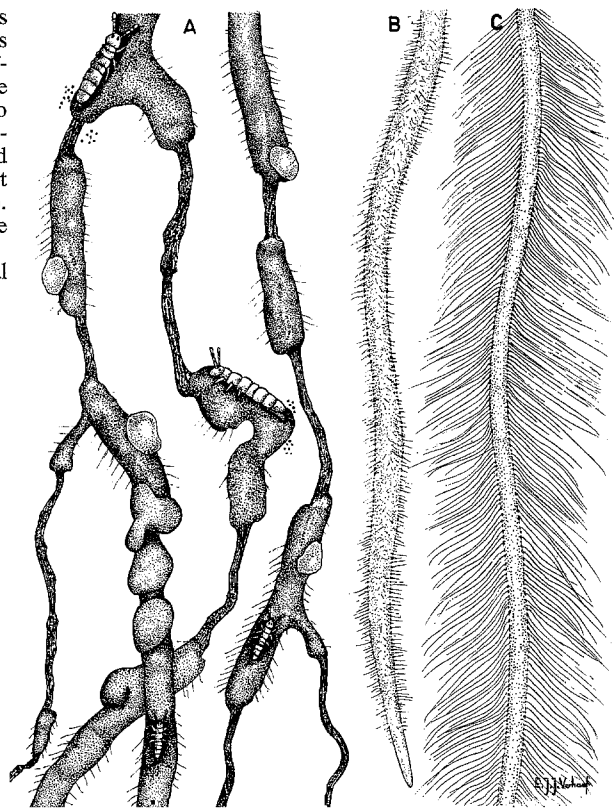

FIG. 2. A: White clover roots with some specimens of *Folsomia quadrioculata* eating the cortex right down to the vessels. The root-knots are indicated and some excrement is drawn near the top. B: Healthy white clover roots. C. Healthy perennial rye grass roots.

cut, had great difficulty in growing again, but even so, it did not die. Later a slight recovery was noted. At the end of the experiment the dry weight of the white clover in this pot was found to be no more than a quarter of that of the plants grown in the control pot where no springtails had been introduced. At that time the majority of the *F. quadrioculata* specimens had died or disappeared.

During the winter of 1962/63 the experiment with the cuvettes was repeated. This time species of *Verticillium*, *Rhizoctonia* and *Cylindrocarpon*, cultivated on soil-oatmeal, were introduced first into the cuvettes. The experiment was again done with ten cuvettes, five of which received no springtails. In the cuvettes with no springtails, the white clover roots seemed in some cases to be slightly affected, but, even so, the plants showed no signs of suffering. In the cuvettes where *F. quadrioculata* was introduced, no fungus infection could be determined, but after a short period the roots showed signs of being eaten, although not to such a great extent as in the first experiment. Here again *F. quadrioculata* preferred to eat the fungi and possibly the bacteria, and then, only when this supply had been exhausted, were the white clover roots eaten.

This observation must not be regarded as a generalisation for other springtails. Among these it might be that there are species which feed on white clover roots, but are not mycophagous and so can cause ports of entry for fungi.

In the pot experiment, before the insertion of the springtails, it became evident, on the basis of the dry weight analysis, that not one of the five fungus species introduced caused damage to the white clover roots. Primarly these fungi are thus not harmful; they need ports of entry, before there is a possibility of causing the plants any damage. The crop analysis after the addition of *F. quadrioculata* showed a somewhat more favourable result for pots containing *Verticillium* or *Fusarium* and springtails in comparison with the control pots and the pots containing only the fungi. In the other pots, i.e. with *Rhizoctonia*, *Tubercularia* or *Cylindrocarpon*, no differences in the white clover yields could be discerned between the plants from the combination of springtails and fungi on the one hand and the fungi alone on the other. Thus the observations from this pot experiment confirmed the results of the cuvette trials.

CONCLUSIONS

In the leys investigated, the same species of springtails were found on both white clover and grass. No evidence was obtained that springtails preferred white clover to grass. In contrast to this, in pot experiments in the greenhouse, a greater number of springtails, especially of *Folsomia quadrioculata*, were determined on the white clover than on the grass. In the summer, *Sminthurinus trinotatus* was usually found and in the winter a species probably closely related to *Onychiurus bicampatus* was abundant.

The species *Folsomia quadrioculata* can feed on white clover roots. Addition of weakly parasitic soil fungi did not lead to the disappearance of the white clover because *Folsomia* preferred feeding on the fungi to the white clover. The springtail is thus capable of making ports of entry for the fungi, but the latter are not able to cause infection themselves. This does not exclude the possibility that anotner combination of soil fauna and flora not yet investigated could affect the white clover sufficiently to cause its disappearance.

ACKNOWLEDGEMENT

The writer is indebted to Mr. W. N. ELLIS, Entomological Department of the Zoological Museum of the University of Amsterdam, for his help in determining the springtail species mentioned in this article.

REFERENCES

ENNIK, G. C., J. KORT, A. M. VAN DOORN & B. LUESINK, – 1962. Bodemparasieten als oorzaak van slechte groei van witte klaver in grasland. Jaarb. I.B.S. 1962: 27–31.

GISIN, H., – 1960. Collembolenfauna Europas. Museum d'histoire naturelle, Genève.