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NOTE ON THE ABILITY OF CERTAIN STRAINS OF RHIZOBI
FROM PEAS AND CLOVER TO INFECT EACH
OTHER'S HOST PLANTS

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The ability of nodule bacteria of the pea and of the clover groups to infect each other's host plants has been claimed (Wilson, 1939), but these two groups have usually been regarded as distinct and have indeed been given different specific names. The existence in each inoculation group of strains serologically related to some from the other group suggested that certain strains having a specific ability to cross-infect may exist in the two groups (Kleczkowski and Thornton, 1944). The following experiments were made to examine this possibility.

INFECTION OF RED CLOVER BY STRAINS DERIVED FROM PLANTS OF THE PEA GROUP

In these tests red clover was grown from externally sterilized seeds on a nitrogen-deficient mineral salts agar medium in test tubes, previously plugged and sterilized in the autoclave. After germination, the cultures were inoculated with the strain to be tested. At Rothamsted, 18 strains of *Rhizobium* from peas, beans, and vetches were tested, using 4 parallel tubes, each containing 3 to 4 clover seedlings, and 4 uninoculated control tubes which remained without nodules. Of the 18 strains, 6 produced totals of from 9 to 22 nodules in the four tubes, and 6 more strains produced from 1 to 4 nodules. In all cases the first appearance of nodules was delayed for about six weeks and the nodules which then appeared were small and conferred no visible benefit to the host plant.

At Glasgow, a similar test was made using 14 strains derived from peas. Of these, only one strain produced nodules on clover. This one, however, produced nodules in five out of six replicates. Six control tubes remained without nodules.

Further tests were carried out with 2 strains that had produced at Rothamsted 14 and 17 nodules, respectively, on red clover. The two strains, B and 313, originally derived from peas by the Wisconsin Agricultural Experiment Station, were selected because their serological behavior towards six antisera was known and because a homologous antiserum against one of them, B, had been obtained. These two strains were reisolated from clover nodules at Rothamsted and inoculated onto pea plants, upon which they readily developed nodules. They were then reisolated from peas and their serological behavior found to be identical with that of the original cultures, which had not been passed through clover.

An independent cross inoculation test was then made at Glasgow with these

two strains, using the original cultures and those that had been reisolated from clover nodules. The nodulation produced at Glasgow in 12 replicates was as follows:

<i>Culture</i>	<i>No. of tubes with nodules on red clover</i>	<i>Total number of nodules</i>
Pea strain B, original	5	11
The same, reisolated from clover	4	11
Pea strain 313, original	10	38
The same, reisolated from clover	9	41

Eight uninoculated control tubes remained without nodules.

The figures show no evidence that passage through clover produced any adaptation increasing the strains' ability to infect this plant.

INFECTION OF PEAS BY STRAINS DERIVED FROM CLOVER

Difficulty has often been experienced in obtaining nodules on peas grown under rigidly aseptic conditions, even after inoculation with bacteria from pea nodules. For this reason, it was thought best to make preliminary cross inoculation experiments with clover nodule bacteria on peas grown in pots of sterilized sand supplied with a nitrogen-deficient culture solution. The risk of air contamination was met by using strains of bacteria that could easily be identified serologically. Eight strains, originally derived from clover nodules, were tested, and of these, three, strains R, C, and Pentyr 4, produced nodules on peas in 6 weeks. Each of these strains was reisolated, passed through clover, reisolated, passed a second time through peas, reisolated, and passed a second time through clover. After this series of passages the three strains were tested against six antisera which, in the case of two of the strains, included their homologous antisera. All three strains were found to give agglutination reactions identical with the original cultures which had not been passed through peas. In this experiment some nodules developed on the control plants, but isolations made from these bore no serological resemblance to any of the three strains, R, C, or Pentyr 4. These three strains were then sent to Glasgow, where they were independently tested on peas. This test was made in large test tubes, containing sand and a nitrogen-deficient culture solution, which were plugged, sterilised, and sown with externally sterilised peas. Eleven uninoculated control tubes were included, and all of them remained free from nodules. Ten parallel tubes were inoculated with each culture. All three strains produced some nodules on the peas, but only in a few of the replicates. As the peas were harvested after 7 weeks, this may have been due to a delay in nodule formation similar to that observed in the reciprocal cross inoculation. Concurrent cultures of peas supplied with strains originally derived from peas developed plentiful nodules. The development of nodules by strains R, C, and Pentyr 4 was as follows:

<i>Culture</i>	<i>No. of tubes with nodules on peas</i>	<i>Total number of nodules</i>
Clover strain R, original culture	1	4
Clover strain Pentyr 4, original culture	3	31
Clover strain C, original culture	0	0
The same, reisolated from pea at Rothamsted	1	4

Isolations from the nodules on plants supplied with strains R and C, and from a single nodule that grew in a tube supplied with strain Pentyr 4, were found to agree in their agglutination reactions with the original cultures. The other 30 nodules in replicate tubes supplied with the last strain were found to be contaminants.

In the same experiment, 14 other strains, obtained from clover nodules, were tested on peas, but of these only 4 strains produced nodules.

Thus the ability of certain strains of *Rhizobium* from clover to produce nodules on peas and of certain strains from peas to infect red clover has been confirmed by independent cross inoculation tests made at Rothamsted and at Glasgow. In these tests, strains were cross-inoculated two or three times and, after reisolation, were found to agree in serological behavior with the original cultures.

The formation of nodules on such cross inoculations, however, took place only to a limited extent, and, at least in the case of pea strains on clover, after an unusually long interval. In no case did the host plant derive visible benefit from the strain belonging to the other inoculation group. The fact that only certain strains will cross-inoculate may explain the disagreement in the findings of other workers.

REFERENCES

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