

# STUDIES ON THE LEAF NODULES VI

## ON THE COMBINED EFFECTS OF HEAT AND ANTIBIOTIC UPON THE LEAF-NODULAR PLANTS IN JAPAN

By Tamotsu YAMADA

(Biological Laboratory, Faculty of Education, Chiba University)

### I. Preface

In order to make the non-leaf-nodulated plants, treat the seeds of the *Ardisia crispa* (Manryo) and *A. punctata* (Karatachibana) with heat or high concentration of antibiotics, appear various effects, especially, not only the non-leaf-nodulated plants but also according to the growth of their seedlings the knobs (Höcker) are made on their growing points to the considerable number of them in proportion to the temperature, concentration, and treating duration, and then their growth stop. But by the same treatment, no knob is made on the non-nodular plants, e.g. the *A. japonica* (Yabukoji), closely affined to them. From this, the knob probable be made by a chemical substance, secreted by leaf-nodule bacteria, as already been reported in the preceding papers by the author.

The author practised the following experiments in order to make clear the combined effects of heat and antibiotic upon the *A. crispa* and *A. punctata*.

It is pleasure to record here a debt of gratitude to Mr. Tomonari for his kindness in photographing.

### II. Experimental Material and Method

The seeds of the *A. crispa* and *A. punctata* were collected in December, 1953 and preserved in dry sand until April, 1954 and were used as material after peeling.

These seeds were sown after treated with warm water of 25-30°C for ten minutes preliminary and dipped them at 50°C for ten minutes in the thermostat bath then soon after immersed them in 500 $\gamma$  solution per cc of streptomycin or terramycin for five days.

### III. Experimental Result

As shown in table 1, in general, the germination percentage was very low compared with control, then in the *A. crispa* it was far higher than the *A. punctata*. The non-leaf-nodulated plants were made 16.67 and 3.64% by the treatments of streptomycin and terramycin respectively in the *A. crispa* but almost of them died within a year, in the other hand, there made non of them in the *A. punctata* by the same treatments.

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Table 1. Experimental result of the combined treatment of heat (at 50°C for ten minutes) and antibiotic (immersed in 500γ solution per cc of streptomycin or terramycin for five days).

| Plant              | Antibi-<br>otic | Germina-<br>tion per-<br>centage | Non-leaf-<br>nodulated<br>plant(%) | Non-foli-<br>ar plant<br>(%) | Average<br>leaf-nodule<br>number<br>on a plant | Average<br>leaf-nodule<br>number on<br>a leaf | Knobbed<br>plant<br>(%) |
|--------------------|-----------------|----------------------------------|------------------------------------|------------------------------|--|---|-------------------------|
| <i>A. crispera</i> | St              | 34.50                            | 16.67                              | 20.37                        | 4.9  | 2.7   | 92.68                   |
|                    | Te              | 33.50                            | 3.64                               | 20.00                        | 4.8  | 3.1   | 95.12                   |
|                    | Cont.           | 85.00                            | 0                                  | 0                            | 8.4  | 3.5   | 0                       |
| <i>A. punctata</i> | St              | 5.00                             | 0                                  | 0                            | not investigated                               |   | 100.00                  |
|                    | Te              | 3.50                             | 0                                  | 12.50                        | not investigated                               |   | 85.71                   |
|                    | Cont.           | 85.00                            | 0                                  | 0                            | not investigated                               |   | 0                       |

St=streptomycin Te=terramycin Cont.=control

The non-foliar plants were made in considerable high percentage by streptomycin and terramycin in the *A. crispera* but on the contrary in the *A. punctata* they were scarcely made 12.50% by terramycin only. The average leaf-nodule number of a plant and a leaf in the *A. crispera* diminished considerably compared with control and the knobbed plants of the both species were made in very high percentage throughout the whole experiments.

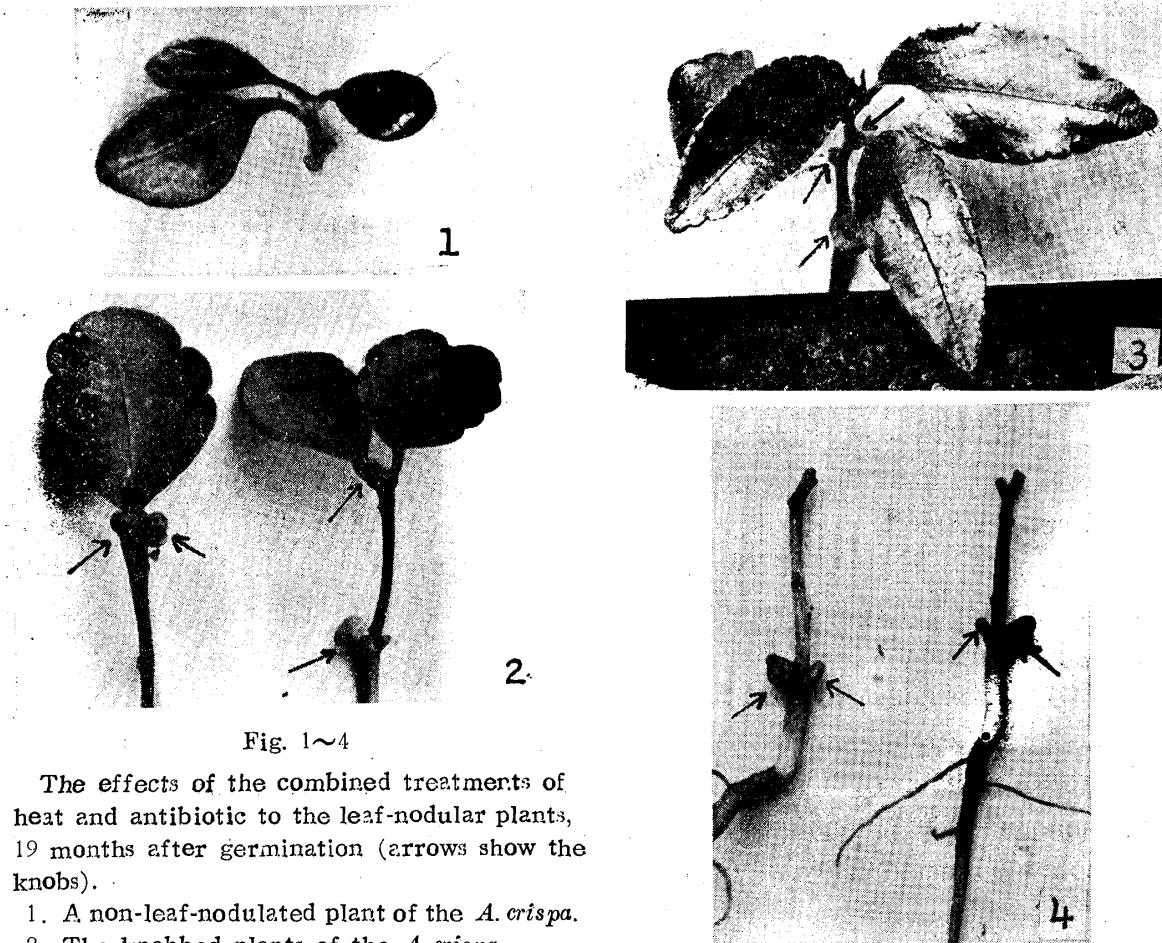


Fig. 1~4

The effects of the combined treatments of heat and antibiotic to the leaf-nodular plants, 19 months after germination (arrows show the knobs).

1. A non-leaf-nodulated plant of the *A. crispera*.
2. The knobbed plants of the *A. crispera*.
3. A knobbed plant of the *A. punctata*.
4. Two non-foliar and knobbed plants of the *A. crispera*.



Fig. 5~6

Normal *A. crispera* (5) and *A. punctata* (6), 19 months after germination.

From above mentioned results, it is clear that the combined effects of heat and antibiotic to the leaf-nodular plants are stronger than the single treatment by each of them and by the combined treatment there made many deformed individuals, namely even they germinate but never develop their leaf or some of these deformed ones never appear on the ground and live in the soil. This particular phenomenon was firstly observed by this treatment.

#### IV. Consideration

Treat the seeds of the *A. crispera* and *A. punctata* combined with heat and antibiotic, the germination percentage becomes lower considerably compared with the single heat treatment (at 50°C for ten minutes) in the *A. punctata*, in the other hand, in the *A. crispera* the non-leaf-nodulated plants are made in high percentage and then the knobbed plants are also made in higher percentage in the both species.

Compared the combined with the single antibiotic treatment, the germination percentage becomes lower in the *A. crispera* but in the *A. punctata* it is slightly higher. The average leaf-nodule number of a plant diminishes in the *A. crispera* and the knobbed plants are made also in higher percentage in the both species.

From above mentioned results, it is evident that the combined effects of heat and antibiotic are stronger than the each single treatment of them, perhaps this may be resulted from the cooperative effects of heat and antibiotic. The peculiar phenomenon that was observed firstly by this combined method was the production of considerable number of non-foliar plants which never develop their leaf after their germination, but this may be reduced also from the stronger action of combined method, and in this case it seems that heat acts more effective than antibiotic generally.

#### V. Summary.

When sow the seeds of the *A. crispera* and *A. punctata* after treated combined with

heat and antibiotic (treat at 50°C for ten minutes and then immerse them for five days in 500 $\gamma$  solution per cc of streptomycin or terramycin), the stronger effects appear than in the case of their each single treatment, namely non-leaf-nodulated, non-foliar, and knobbed plants are made in higher percentage. Probably this may be resulted from the effects of the cooperation of heat and antibiotic, then heat seems in the tendency of more effective than antibiotic.

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References

1. Hanada, K. : Ueber die Blattknoten der *Ardisia*-Arten. Isolierung der Bakterien und ihre Stickstoffbindende Kraft in Reinkultur. Jap. Jour. Bot., Vol. 14, No. 2, 1954.
2. Miede, H. : Weiter Untersuchungen über die Bakteriensymbiose bei *Ardisia crispa*. II Die Pflanzen ohne Bakterien. Jahrb. f. wiss. Bot., Bd., 58, 1919.
3. Yamada, T. : Studies on the leaf nodules. I. On the historical researches of the leaf nodules. Bull. Fac. Edu. Chiba Univ. Vol. 3, 1954.
4. Yamada, T. : Studies on the leaf nodules. II. On the leaf nodules in the genus *Ardisia*. Bull. Fac. Edu. Chiba Univ. Vol. 2, 1953.
5. Yamada, T. : Studies on the leaf nodules. III. Further studies on the effect of heat treatment upon *Ardisia crispa* and *A. punctata*. Bull. Fac. Edu. Chiba Univ. Vol. 4, 1955 (a).
6. Yamada, T. : Studies on the leaf nodules. IV. Formation of the leaf nodules as affected by antibiotics. Bull. Fac. Edu. Vol. 4, 1955 (b).
7. Yamada, T. : The growth and chlorophyll formation in seedlings as affected by antibiotics. Bot. Mag. Tokyo, Vol. 66, No. 781-782, 1953.
8. Yamada, T. : Studies on the leaf nodules V. The formation of knobs by the effect of antibiotics on the *Ardisia crispa* and *A. punctata*. Bot. Mag Tokyo, Vol. 68, No. 808, 1955.
9. Yamada, T. : 高濃度抗生物質混合液の菜種幼植物に及ぼす影響 科学 Vol. 24, No. 2, 1954.
10. Yamada, T. : 抗生物質による菜種幼植物の色素. 発現 科学 Vol. 24, No. 12, 1954.
11. Hori, K. : 葉瘤植物に及ぼす熱及び抗生物質の影響. 科学 Vol. 25, No. 11, 1955.