SOME STUDIES ON THE *PSEUDOMONAS*

PRUNI E. F. SMITH.

BY

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(鐵塚喜久治：—李黒斑病細菌の研究)

The study of the *Pseudomonas pruni* has chiefly been made in the U. S. of America by many investigators since the first work of Smith (1) published in 1903. In Japan it has also been described as black spot of the plum or bacterial disease of stone fruits. The writer of this article reported of the serious outbreak of this disease on the peach in Shizuoka and published some studies connected with it in a preliminary paper (2).

Since then the writer has made some inoculation experiments on the plum and peach as well as on many species of genus *Prunus* and on the many other rosaceous plants—sixteen genera including forty-four species. Furthermore the writer ascertained the influence of the soil moisture upon the infection of causal organisms.

I. INOCULATION EXPERIMENT TO PLUM FRUITS.

A number of strains of causal organism were isolated from various sources; such as the leaf, branch and fruit of diseased peach and plum tree, and their virulence was proved by many inoculation experiments either on the normal leaves or fruits. It was thus proved that these several strains cause not only the shot-hole disease on the leaf but also the black spot on the plum fruit. The following table shows the results of an experiment of the spraying inoculation to plum fruits (Terada variety) which was carried out at Okitsu Horticultural Experiment Station, Shizuoka Prefecture, during May and June 1919.

Table I. The results of inoculation to plum fruits.

Strains.	Date of inoculation.	No. of inoculated fruits.	No. of infected fruits.
Control	May 28	10	0
A	"	10	9
C	"	10	10
A	June 7	10	10
C	"	10	8
B	June 23	10	7
C	"	10	8
D	"	10	7

Remarks; The organisms used as inoculants were cultured on the agar slant medium for 4 days at 30° C. in each case. The strain noted by A is one which had been isolated from the peach fruit, July 19, 1918; B from a peach branch, Oct. 23, 1918; C from a hibernated plum branch, March 17, 1919, and D from a plum fruit, June 8, 1919, respectively.

From the above table it is observed that, with the strains of B and C which had been isolated from infected branches in early spring or autumn, the infection occurred as well as with those of A and D. This fact suggested that the viability of the causal organism in the infected tissue of a twig hibernated through the winter under field conditions, is not destroyed and serves as a central source of infection in the spring.

II SUSCEPTIBILITY OF ROSACEOUS PLANTS TO *PS. PRUNI*.

The plants described as the host of *Ps. pruni* up to the present are the following.

1. Apricot. (*Prunus Armeniaca* L. var. *Anzu* Max.)
2. Nectarin. (*Prunus Persica* St. var. *necturina* Max.)
3. Peach. (*Prunus Persica* S. Z.)
4. Plum. (*Prunus domestica* L.)
5. Wragg Cherry. (*Prunus Cerasus* L.)

It has been ascertained that there are various degrees of susceptibility and resistance among the different varieties of these plants, and F. M. Rolfs (3) especially mentioned the difference of natural infection in many varieties of the first four kinds, while the wragg cherry was discovered by W. G. Sackett as a new host.

Inoculation experiments have been made on different species of rosaceous plants

in College of Agriculture, Komaba, Tokyo, with the pure culture of causal organisms. The experiments were repeated three times, the first on May 20, the second May 31, and the third June 19, 1919.

The organisms used are five days old culture on the agar medium from the strain C. As the host, two healthy branches were chosen from each plant, and in the first two experiments they were covered with a paraffin-paper bag and the branches within the bag were sprayed with organisms suspended in sterilized well-water. In the last experiment the inoculation was made in the tissue of the branches at three points each by means of hypodermic-syringe and then covered with a paraffin-paper bag.

Observation were made two to four weeks after inoculation, in order to ascertain whether the disease had attacked the plant or not. The results obtained are represented in the table II.

Table II. The results of inoculation experiment to rosaceous plants.

Names. of Plants.	1	2	3	
	leaf.	leaf.	leaf.	stem.
<i>Ameranchier asiatica</i> Endl.	—	—	—	—
<i>Chaenomeles japonica</i> Lind.		—	—	—
<i>Chaenomeles lagenaria</i> Koidz.		—	—	—
<i>Crataegus cuneata</i> S. et Z.	—	—	—	—
<i>Cydonia sinensis</i> Thouin.	—	—	—	—
<i>Cydonia vulgaris</i> Pers.		—	—	—
<i>Eryobotrya japonica</i> Lind.	—	—	—	—
<i>Kerria japonica</i> DC.	—	—	—	—
<i>Malus floribunda</i> Sieb.	—	—	—	—
<i>Malus pumila</i> Mill. var. <i>domestica</i> C. K. Sah.	—	—	—	—
<i>Malus Zumi</i> Koidz.	—	—	—	—
<i>Photinia glabra</i> Max.	—	—	—	—
<i>Photinia villosa</i> DC.	—	—	—	—
<i>Pirus communis</i> Th.	—	—	—	—
<i>Pirus serotina</i> Rehd.	—	—	—	—
<i>Prunus Armeniaca</i> L. var. <i>Anzu</i> Max.	+	+	+	+
<i>Prunus avium</i> L.	—	—	—	+

<i>Prunus Buergeriana</i> Miq.	-	-	-	+
<i>Prunus cerssipes</i> Koidz.	-	-	+	+
<i>Prunus domestica</i> L.	+	+	+	+
<i>Pr. donarium</i> Sieb. subsp. <i>elegans</i> Koidz. var. <i>glabra</i> Koidz.	-	-	-	+
subsp. <i>speciosa</i> Koidz.	-	-	-	-
<i>Prunus Itosakura</i> Koidz.	-	-	-	-
<i>Prunus japonica</i> Thunb.	+		+	+
<i>Prunus Mume</i> S. et Z.	+	+	+	+
var. <i>Microcarpa</i> Mak.		+	+	+
var. <i>Bungo</i> Mak.		+	+	+
<i>Prunus Persica</i> S. Z.	+	+	+	+
(gard. var. <i>Early Rivers</i> .)	+	+	+	+
(gard. var. <i>Danjuro</i> .)	+	+	+	+
var. <i>nectarina</i> Max. (gard. var. <i>Early Newington</i> .)	+	+	+	+
<i>Prunus tomentosa</i> Thunb.	-	-	-	-
<i>Prunus triflora</i> Roxb.	+	+	+	+
<i>Prunus yedoensis</i> Matsum.	-	-	-	-
<i>Raphiolepis umbellata</i> Mak.	-	-	-	-
<i>Rosa Banksiae</i> R. Br.		-	-	-
<i>Rosa laevigata</i> Michx.	-	-	-	-
<i>Rosa microphylla</i> Roxb.	-	-	-	-
<i>Rosa multiflora</i> Thunb.	-	-	-	-
<i>Rubus Kinashii</i> Lev. et Vnt.	-	-	-	-
<i>Rubus Thunbergii</i> Sieb. et Zucc.		-	-	-
<i>Rubus Tokkura</i> Sieb.		-	-	-
<i>Rubus trifidus</i> Thunb.	-	-	-	-
<i>Rubus triphyllus</i> Thunb.		-	-	-
<i>Sorbus japonica</i> Koehne.	-	-	-	+
<i>Spiraea cantoniensis</i> Lour.	-	-	-	-
<i>Spiraea japonica</i> L.	-	-	-	-
<i>Spiraea Thunbergii</i> Sieb.			-	-
<i>Stephanandra incisa</i> Zab.	-	-	-	-

From the results of the experiment it seems certain that almost all species of the genus *Prunus* both cultivated and wild, and the *Sorbus japonica* also, are liable to be attacked by *Ps. pruni*. The following are the plants newly proved to be susceptible to the disease, Namely; *Prunus Buergeriana*, *P. cerssipes*, *P. donarium* subsp. *elegans* var. *glabra*, *P. Itosakura*, *P. japonica*, *P. Mume*, *P. Mume* var. *mi-*

crocarpa, *P. Mnme* var. Bungo, *P. triflora* and *Sorbus japonica*.

When the inoculations are made on the stems of the genus *Prunus*, there appear generally some canker-like or sunken parts, while on the *Sorbus japonica* small gall, or knobs are developed at the point of inoculation.

All these affected parts have been examined microscopically to determine the presence of the bacteria within the tissue.

III. INFLUENCE OF SOIL MOISTURE UPON INFECTION.

It is well known that there is close relation between the infection of parasites and the environmental conditions; temperature, humidity, light etc. It was mentioned by E. F. Smith (I) that the occurrence of stomatal infection is due to the nature of this organism, and it has been recognized by many successive investigators as a typical example of the organisms which show the stomatal infection. It is, therefore, to be noted that the stomatal condition exerts a remarkable influence on the infection of this organism. Concerning the opening and closing of the stomata, however, it is difficult to give a decisive opinion, because it is controlled by complex factors of the environment, but as the stomatal condition is connected to no small extent with the transpiration, so the soil moisture must have an important relation with the occurrence of the disease.

The writer has made an experiment to ascertain the effect of soil moisture upon the infection of this organism. For the experiment, there were prepared in a green house, nine pots of Early Rivers peach trees of two years old; three of them were kept always in abundance of moisture, the pots being placed in a shallow vessel, in which the water stood a half inch in depth constantly. Whereas the other six pots were kept in a lowest moisture possible consistent with life, namely were sparingly watered once a week.

Inoculation was made on June 4 by means of spraying with five days old agar cultures of causal organisms, which were suspended in sterilized well-water, care being taken especially to spray the lower surface of leaves. After inoculation some were covered with paraffin-paper bag and the other not covered, and as a control a few branches of each plant were left unsprayed. Inoculated branches were sprayed with water, every day, for a week in order to furnish sufficient humidity.

Observations were made four weeks later counting the number of diseased spots on every leaf, and the result obtained are given in table III.

Table III. The results of the inoculation experiment upon the influence of soil moisture.

Soil condition	No. of pot	Condition of branch.	No. of branches.	No. of leaves.	Total no. of spots	Averaged no. of spot per leaf.	Means of spots in covered.	Means of spots in not-covered
MOISTURED POTS.	I	Covered	1	9	139	15.444	10.456	3.709
		Not-covered	6	57	209	3.666		
		Control	5	62	0			
	II	Covered	1	6	16	2.666		
		Not-covered	5	33	133	4.030		
		Control	2	21	0			
	III	Covered	2	26	344	13.230		
		Not-covered	2	37	127	3.432		
		Control	2	30	0			
DRIED POTS.	IV	Covered	1	15	37	2.466	0.696	0.204
		Not-covered	1	14	15	1.071		
		Control	1	10	0			
	V	Covered	1	7	5	0.714		
		Not-covered	1	5	0	0.0		
		Control	0					
	VI	Covered	1	12	7	0.483		
		Not-covered	8	64	4	0.062		
		Control	2	19	0			
	VII	Covered	2	15	3	0.200		
		Not-covered	4	32	0	0.0		
		Control	1	15	0			
	VIII	Covered	1	20	3	0.150		
		Not-covered	3	39	1	0.025		
		Control	0					
	IX	Covered	2	48	3	0.062		
		Not-covered	2	16	1	0.062		
		Control	1	14	0			

From the table III it will be seen that the soil moisture played a potent role in the infection of the leaf-spot bacteria, and that it was far more evident than the atmospheric moisture.

Therefore it is probably advisable that in orchard having high level of underground water to look carefully after the drainage which is an important point in the prevention of this disease.

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摘 要

李の黒斑病細菌 (*Pseudomonas pruni* E. F. Smith) は我國に於ても李、桃等の葉に穿孔病を起すのみならず、桃の果實に七月頃發生して褐色裂傷狀の病害を起すものなり。

本細菌は又莖を侵すものにして特に李に最も多し、潰瘍狀の病患部を生じ、細菌は其病組織内に越冬して翌春傳播の中心となるものなり。即ち十月下旬桃の枝より、或は三月中旬發芽前の李枝より分離せる菌も夏季果實より分離せるものと同様に感染發病する事を確め得たり。

本細菌の寄主として今日迄記載されたる植物は、アンズ、ズバイモモ、モモ、スモモ、スミセイヤウミザクラの五種なりき。大正八年、東京帝國大學農學部植物園に於て十六屬、四十餘種の薔薇科植物に就て接種試験を行ひたる結果によれば上記の五種のみならず、スモモ屬の植物には殆ど總てに感染し得るものの如し即ち更にイヌザクラ、チャウデザクラ、ヤマザクラ、ヒガンザクラ、ニハウメ、ウメ、コウメ、ブンゴウメ、ヨネモモ、等のスモモ屬植物に陽性の結果を得たり。

スモモ属以外の植物にては單にナナカマドの莖に感染（組織内に注射接種の場合）し小腫起物を形成せり。

土壤濕度の多少と葉部感染との關係に就ては更に實驗を重ねべきも、第一回試験の結果によれば土壤濕度の高き場合は比較的乾燥せる場合よりも、葉に於ける本菌感染の度、即ち穿孔を生ずる割合著しく大なり。