Studies on the Varietal Resistance of Sweet Potato to the Root-knot Nematode Injury

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CONTENTS

I. Introduction

As is well known, the root-knot nematode, *Heterodera marioni* (Cornu, 1879) is a cosmopolitan pest having a wide host range. In 1917 it was first discovered by Elliott (1918) in the United States of America to attack sweet potato. Nineteen years later, some females of this nematode were occasionally detected at Makurazaki, Kagoshima Prefecture, while in examination of raw slices of tubers cut for milling in cooperation with the late Mr. K. Sakai, the entomologist of the Kagoshima Agricultural Experiment Station. Under this circumstance field survey was undertaken, revealing that this pest was quite prevalent in sweet potato growing areas in Japan, especially of sandy soil and of a great economic importance in relation to the production of potato.

The fact that there is a marked varietal difference in injury of sweet potato caused by the nematode in question was already put on record by Weimer and Harter (1925) and Poole and Schmidt (1927). This state of things holds good in Japan, according to investigations made since 1941 at the Experiment Stations of Kagoshima and Chiba, Now several resistant varieties selected are gradually substituted for the susceptible ones in the seriously infested areas.

The present account is based upon some series of experiments attempted with a view to elucidating why a considerable difference exists among varieties of sweet potato in the extent of the root-knot injury.

In this opportunity, the writer wishes to express his hearty thanks to Prof. Dr. T. Kaburaki for the kindness in reading through the manuscript. It might be also mentioned that this work was prosecuted by the grant-in-aid from the Ministry of Education.

II. Examination of resistance of sweet potato varieties to the root-knot nematode in Kagoshima

In order to select the resistant varieties of sweet potato to the root-knot nematode, experiments were conducted in cooperation with

Table 1.

Degree of gall formation in different varieties

Varieties	1944 Plots		1945 Plots		1946 Plots	
	A	B	A	B	A	B
Norin No. 1	+++	++++	+++++	++++	+++++	+++++
〃 No. 2	−	−	−	−	±	±
〃 No. 3	+	+	±	±	+	±
〃 No. 7	+	+	+	++	+	±
Kyushu No. 1	....	....	....	....	++++	+++++
〃 No. 3	++	+	++	++	++	+
〃 No. 8	+	+	++	+++	++	++
〃 No. 10	....	....	....	....	++++	++++
〃 No. 12	....	....	++	++++	....	....
Okinawa No. 100	+++	+++	+++	++++	....	....
Kokei No. 3	+++	+++	+++	++++	....	....
Gokoku	+++	+++	++++	++++	....	....
Oiran	++++	++++	++++	+++++	....	....
Shichifuku	++++	++++	++++	++++	....	....
Tsurunashi-genji	++++	++++	+++++	+++++	....	....
Ibaraki No. 1	....	....	....	....	+	....
Taihaku	....	....	....	....	+	±
Hayato	....	....	....	....	++++	+++
Genji	++++	++++	+++++	+++++	+++++	+++++

Note: −, ±, +, ++, +++, ++++, +++++, mark various degrees of gall formation, such as none, rare, very few, few, moderate, many, and very many respectively.

the late K. Sakai at the heavily infested field of Mikasa-mura, Izumi-gun, Kagoshima Prefecture from 1944 to 1946 inclusive. Through the courtesy of the owner, T. Sakamoto, the experimental plots were located on the same field for three years. Early in June slips of different varieties were planted in duplicate plots. The plots were dug at the beginning of November, at which time observations were made on the degree of infestation on the roots of each plant and the yield of each variety. The degrees of gall formation are merely shown in Table 1.

In the most heavily infested plant, vines and leaves did not grow thick, shrinking as if suffering from "dwarf disease", and a small number, or sometimes none, of fibrous rootlets and potato tubers were found, while long thick roots called "octopus' legs" because of their wirty appearance were superior in number. Badly infested tubers were of an irregular shape with a roughened surface. In certain varieties root-skin became decayed and torn. The yield of potato, however, was not always in correspondence with the degree of infection as described by Collins and Hagen (1932) with pineapple. Even in the Kyushu No. 10, a susceptible variety, a fair crop was produced.

III. General behavior of the root-knot nematode in sweet potato

With a view to making out the general behavior of the root-knot nematode investigations were prosecuted with a susceptible variety of sweet potato. The results will be briefly given below.

Larvae enter the root system of the host at the portion close to the root tip. When the larvae assume the final position, they have usually, but not always, a centripetal situation, inserting their head between cells of the plerome and remaining the posterior portion of the body in the periblem and, as the root develops, in the cortex. The enlarging female body pushes the parenchyma of the cortex aside until its posterior end breaks the gall epidermis, so that a gelatinous egg-sac is always secreted outside the gall. The newly hatched larvae escape from the egg-sac into the soil and migrate to, and enter, new root tips. This state of things is similar as that observed in other hosts by some authorities (Byars 1914, Godfrey 1931, Steiner 1934, Christie 1936, etc.).

Under the climatic conditions at Kagoshima, the minimum time needed for the development of the parasite from its entry to the egg-producing stage is about 3 weeks in summer and 4 weeks in autumn, and as many as 5 generations may develop during a season of sweet potato cultivation (from June to October), one generation being 30 days or more on an average.

Galls are classified into three types according to their location on the roots: terminal, intermediate and basal. The terminal galls result from check in further growth of the root tip by the infestation. The intermediate ones, amounting to 80–90% of the galls present (Table 2), are formed when the root tip counteracts and repairs the effect of parasites through additional tissue growth. The basal ones which are found at the base of lateral roots denote a particular case where parasites enter the tip which has only just come out.

Table 2.

Percentage of the three types of galls

Varieties	Basal		Intermediate		Terminal		Total	
	No.	%	No.	%	No.	%	No.	%
Yoshida	42	5.2	733	89.1	48	5.8	823	100
〃	44	10.2	359	83.3	28	6.5	431	100
Genji	13	7.2	149	82.2	19	10.6	148	100

IV. Experimental work and discussion

A. Source of inoculum

Steiner (1925) has pointed out cases of host specialization and host preference in plant parasitic nematodes, inclusive of the root-knot nematode. In view of such possible differences in various populations of the nematode in question, preliminary tests of different sources of inoculum were made. In these tests the potted sweet potato varieties known of degree of resistance were inoculated with newly hatched larvae from galls of such various plants as tomato, cucumber, eggplant and kidney bean respectively. In consequence, each variety exhibited a tendency similar in the gall development to the case inoculated with the larvae whose parents lived on sweet potato. This similarity was also true of sweet potato varieties of different susceptibility planted in such fields as Swiss chard, carrot and Irish potato suffered previously from the injury concerned. Furthermore, there was no evidence that the larvae of sweet potato origin preferred the said crop rather than tomato, eggplant and other plants. Consequently it will be safely stated that there is no host specialization of the root-knot nematode, so far as concern experiments just mentioned. For this reason the writer used larvae from any galls ready to his hand as a source of inoculum in the present work.

Worthy of notice is here that Nozu (1940) puts on record two varieties of sweet potato, Genji and Shichifuku, as unaffected crops at Shimane, though these varieties belong to a susceptible group at Kagoshima and other districts. It is advisable to reexamine these varieties at Shimane.

B. Method of preparing nematode larvae for inoculation in quantities

The method adopted consists in the following procedure. Egg-sacs which protrude beyond the surface of galls on roots of such plants as mentioned in the preceding section are stripped off and arranged in a circle on a watch-glass filled partly with water so as to come just contact with the water. Then the watch-glass is placed in a petri dish containing sufficient water to maintain saturated humidity. After a few days a multitude of newly hatched larvae, being released from the egg-sacs, crowd in the centre of the glass. Thus sufficient quantity of larvae to serve for inoculation is obtained.

C. Method of investigating galls and parasites within

A pot or dish with a plant to be examined is submerged in a water tank and shaken gently till the root system is separated from soil or sand. Separation over, the root system is washed by means of a stream of water with the aid of a hair-pencil until free from adhering soil particles, and then galls are sought with unaided eyes or under magnification.

Parasites within the gall are examined by dissecting under a microscope of 20 magnifications. If necessary, however, the galls, after Godfrey (1935), are fixed with Flemming's strong killing fluid, washed, dehydrated and immersed in clove oil. By this means the black-stained parasites, leaving their position as it is, are distinctly observed through the gall tissue under a microscope.

D. Has the root-knot nematode an ability to distinguish between different varieties of sweet potato?

It has been a prevailing opinion that the resistance of the plant to the nematode is ascribed to the host selection of the parasite. The writer also supposed at the start that the root-knot injury takes place on such a variety as its roots secrete some chemical substance attracting the nematode larvae, and that if different varieties of known degree of resistance are planted together in inoculated soil and left to the selection of the parasite, the susceptible varieties may attract the majority of the parasites, thus reducing the amount of infection in the less susceptible ones. On such an assumption the following experiment was undertaken.

Experiment I. The varieties of sweet potato employed in this experiment were one resistant variety, Norin No. 2, and two susceptible ones, Genji and Yoshida (of Chiba but not of Okinawa origin). One vine-cutting of each variety was planted in an equilateral triangle in each of two pots (14 cm in diameter and 15 cm in depth) on Oct. 2nd, 1946, which was inoculated with several galls from tomato at the centre. On Dec. 12th, the number of galls on the roots of each variety was examined. The results obtained are shown in Table 3.

Table 3.

Number of galls observed on the roots of three varieties planted together in a pot

Pot No.	Varieties		
	Norin No. 2	Genji	Yoshida
1	21	93	651
2	0	0	396

Glancing at this table it is evident that the number of galls on Yoshida is by far greater than those on others, though the data of two pots are not entirely parallel. As such remarkable differences take place under the conditions that the roots of three varieties are entangled with one another, it may be natural to consider that the nematode is able to discriminate a preferable host from others.

In order to ascertain whether this conclusion is reasonable, Experiment II was prosecuted, employing varieties other than those in Experiment I.

Experiment II. The varieties used were two resistant ones, Norin No. 2 and Taihaku, and two susceptible ones, Tsurunashi-genji and Okinawa No. 100. On August 6th, 1949, four vine-cuttings, one for each variety, were set in a square in a petri dish (16 cm in diameter) containing sand moistened with water. On August 10th, inoculation was made at the centre of the dish with the larvae from kidney bean. Such dishes were set up in triplicate. When a period from 11 to 13 days elapsed after inoculation, the number of galls and parasites within was counted with the results, as given in Table 4.

Table 4.

Number of galls and nematodes observed on the roots of four varieties planted together in a petri dish

Varieties	Dish No.	Number of galls	Number of nematodes at different stages					Date of observation
			Larval stage			Adult female	Total	
			1st	2nd	3rd			
Tsurunashi-genji	1	9	1	2	3	7	13	August 21
	2	25	6	32	6	16	60	〃 22
	3	80	29	57	28	35	149	〃 23
Okinawa No. 100	1	13	23	7	1	0	31	〃 21
	2	8	1	5	2	0	8	〃 22
	3	81	32	57	19	11	119	〃 24
Norin No. 2	1	6	6	0	0	0	6	〃 21
	2	5	5	0	0	0	5	〃 22
	3	1	3	0	0	0	3	〃 24
Taihaku	1	0	0	0	0	0	0	〃 21
	2	0	0	0	0	0	0	〃 22
	3	0	0	0	0	0	0	〃 24

Note: Daily temperature during the experiment was about 28.5°C on an average.

A close examination of the table reveals that the same conclusion as in the preceding experiment may be drawn, so far as the quantity of the parasite is concerned. There is, however, one point to which our attention must be paid, and that is the remarkable difference of developmental rapidity of parasites in different varieties: in the susceptible ones some of parasites have already attained to the adult stage, whereas in Norin No. 2, a resistant one, all still remain at the first larval stage, despite of lapse of the same period after inoculation.

Thus the following comes into question.

E. Is a gall the inevitable effect of the nematode infection?

In general a gall is the only apparent symptom of the root-knot nematode infection. It is doubtful, however, whether there are varieties which show no symptom notwithstanding the entry of the nematode, as Steiner (1927) observed with cyclamen. In order to shed light on this question, Experiment III was carried out.

Experiment III. The following procedure was planned, employing the same varieties as those in the preceding experiment. With every variety, a short vine having two adventitious roots in proper length which had started in water was cut off. These vines were radially arranged on a watch-glass (10 cm in diameter) so that the root tips of different varieties might intersect one another, as shown in Fig. 1.

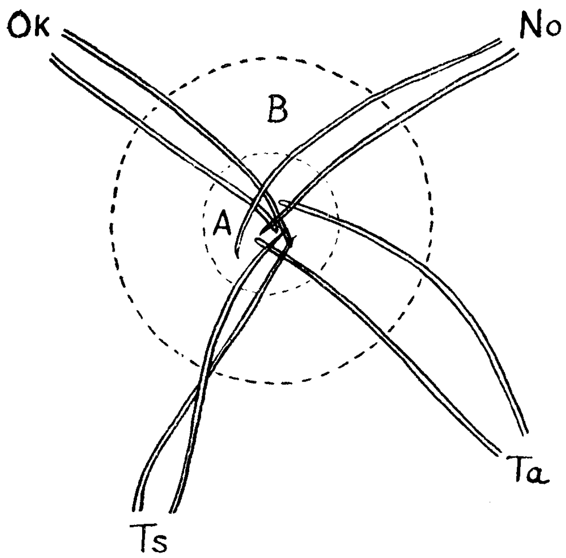

Fig. 1. Arrangement of roots of four varieties (Ok: Okinawa No. 100, Ts: Tsurunashi-genji, Ta: Taihaku, No: Norin No. 2) on a watch-glass. Larvae were inoculated at the portion A and the portion B was covered with fine, moistened sand.

Inoculation was made by applying a small drop of water containing a number of larvae from kidney bean to a portion of intersection of the root tips. The roots were then covered with fine sand, which was moistened afterwards with water. The watch-glass was then placed in a covered, moistened petri dish to prevent the sand from drying. When an interval of 42 hours passed after inoculation, the nematodes within every root were observed by means of the Godfrey's staining method already mentioned.

Up to the time of investigation, no apparent symptom of infection was observed in any variety (Fig. 2). Nevertheless, as shown in Table 5, the roots of both

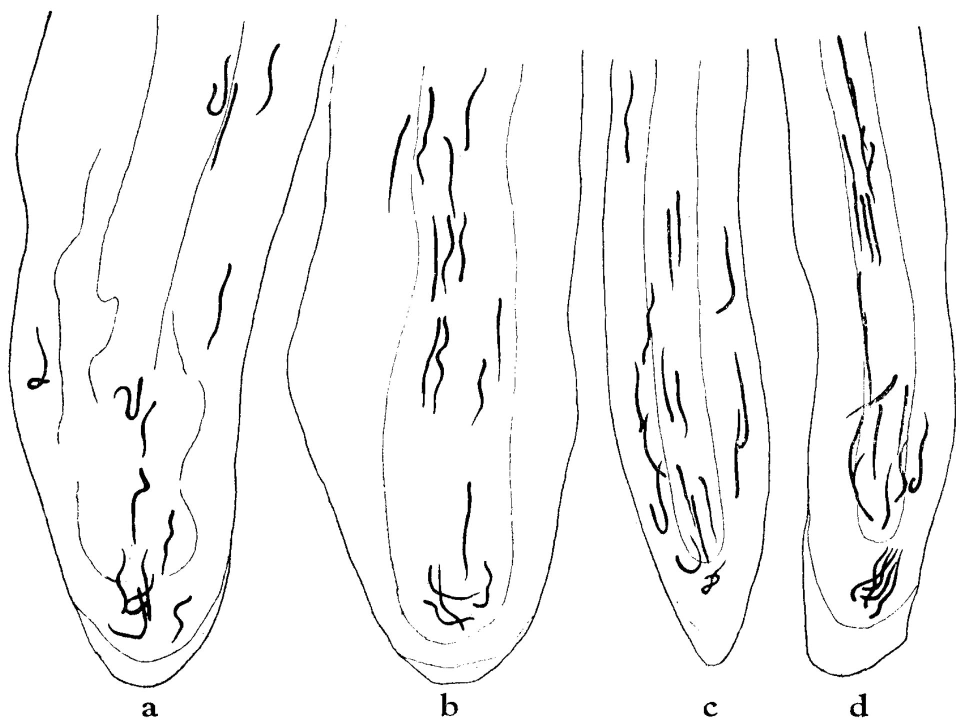

Fig. 2. Nematode larvae found in the roots of four varieties (a: Taihaku, b: Okinawa No. 100, c: Norin No. 2, d: Tsurunashi-genji) at the end of 42 hours after inoculation.

the resistant and the susceptible varieties, without exception, harboured a certain number of larvae. According to a statistic examination, there was no significant difference among the numbers in question of different varieties at 5% level. Therefore, the larvae proved to enter the roots, irrespective of the varieties.

Table 5.

Number of nematodes found in the roots of four varieties at the end of 42 hours after inoculation

Root No.	Tsurunashi-genji	Norin No. 2	Okinawa No. 100	Taihaku
1	23	3	46	12
2	27	19	3	27

The similar fact was also confirmed by Experiment IV which was conducted in a larger scale. The detail of the experiment will be described below.

Experiment IV. *Series A.* The following ten varieties of known degree of resistance were used.

Resistant: Norin No. 2, Norin No. 3, Norin No. 5, Norin No. 7, Norin No. 9 and Taihaku.

Less resistant: Norin No. 4 and Norin No. 8.

Susceptible: Norin No. 1 and Norin No. 6.

Five varieties selected at random from the varieties mentioned above were planted in one petri dish (16 cm in diameter) containing sand, and the remaining five in another dish. On planting, one vine-cutting for each variety was disposed so as to form an equilateral pentagon as a whole, disposition also being determined by a random method. In this manner five pairs of dishes were prepared in total. Every dish was inoculated with larvae from kidney bean at its centre on Sept. 3rd, 1949, and at the end of 10 to 15 days after inoculation, every root of each variety, regardless of symptom of infection, was dissected to examine the parasite within.

Series B. The following ten varieties other than those in Series A were employed.

Resistant: Kyushu No. 5, Kyushu No. 13 and Ibaraki No. 1.

Less resistant: Kyushu No. 15, Okinawa No. 100, Kokei No. 3 and Gokoku.

Susceptible: Tsurunashi-genji, Oiran and Shichifuku.

The general procedure was the same as in Series A, except that inoculation was made with larvae from eggplant on Oct. 24th, 1949, and examination of parasites was made on Nov. 4th to 7th.

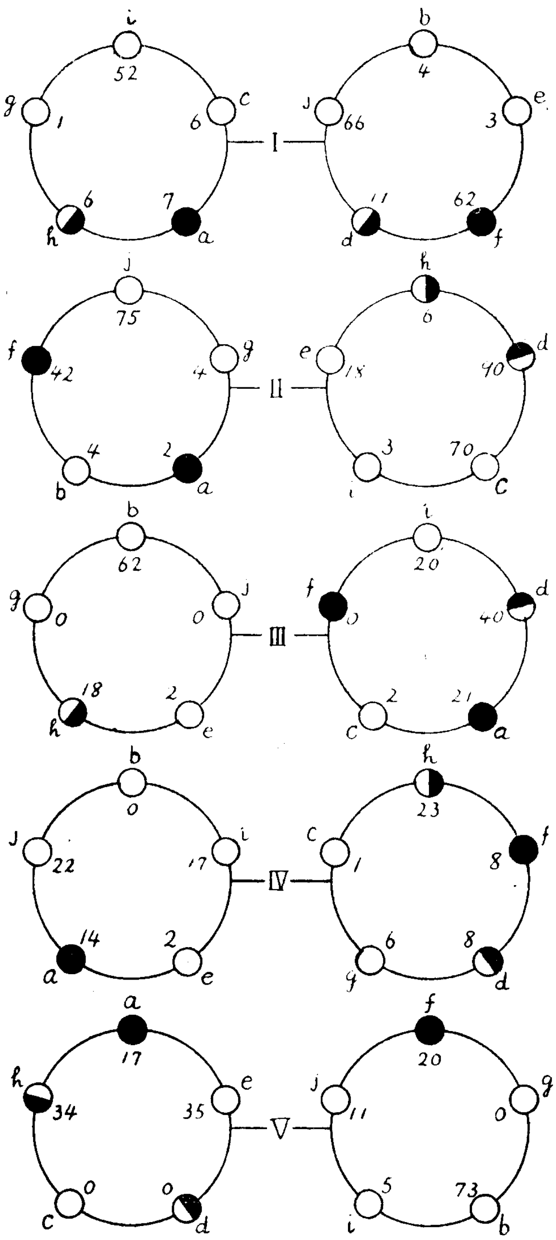

Fig. 3. Distribution and arrangement of varieties and the number of nematodes observed in the roots of each variety ... Series A. a: Norin No. 1, b: Norin No. 2, c: Norin No. 3, d: Norin No. 4, e: Norin No. 5, f: Norin No. 6, g: Norin No. 7, h: Norin No. 8, i: Norin No. 9, j: Taihaku. ○: resistant variety, ◑: moderate one, ●: susceptible one. Numerals designate the number of nematodes observed in the roots.

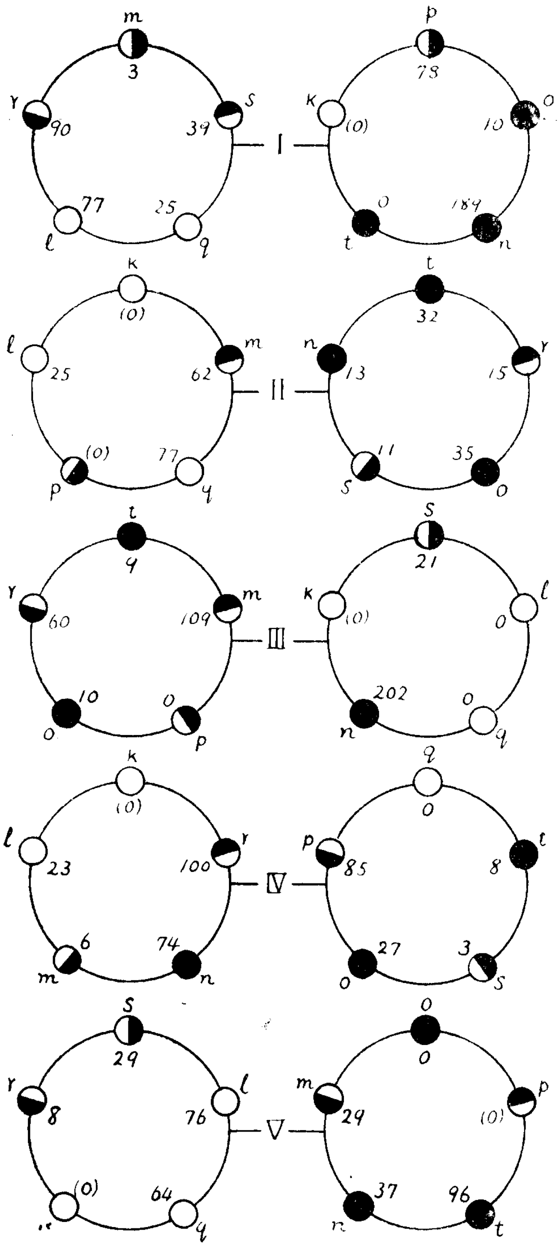

Fig. 4. Distribution and arrangement of varieties and the number of nematodes observed in the roots of each variety.. Series B. k: Kyushu No. 5, l: Kyushu No. 13, m: Kyushu No. 15, n: Tsurunashi-genji, o: Oiran, p: Okinawa No. 100, q: Ibaraki No. 1, r: Kokei No. 3, s: Gokoku, t: Shichifuku. Other remarks are the same as those of Series A, but (0) designates zero due to the absence of the available roots for entry of the nematode.

Results obtained in both series were summarized in Table 6 and Figs. 3 & 4.

Table 6.

Total number of nematodes observed in roots of five plants of each variety

Series A			Series B		
Varieties	Degree of resistance	Number of nematodes	Varieties	Degree of resistance	Number of nematodes
Norin No. 1	S*	61	Kyushu No. 5	R	—**
〃 No. 2	R	143	〃 No. 13	R	201
〃 No. 3	R	79	〃 No. 15	LR	209
〃 No. 4	LR	149	Tsurunashi-genji	S	515
〃 No. 5	R	60	Oiran	S	82
〃 No. 6	S	132	Okinawa No. 100	LR	163**
〃 No. 7	R	11	Ibaraki No. 1	R	166
〃 No. 8	LR	87	Kokei No. 3	LR	273
〃 No. 9	R	97	Gokoku	LR	131
Taihaku	R	174	Schichifuku	S	145
Total		993	Total		1885

Notes: (1) * R, LR and S represent resistant, less resistant and susceptible respectively.
(2) Average temperature during the experiment was 28°C in Series A and 19°C in Series B respectively.
(3) In Series B, owing to low temperature the development of roots was generally retarded. Especially, every plant of Kyushu No. 5 and two of Okinawa No. 100 (**) had no sufficiently long roots to be attacked by nematodes.

On glancing at each individual dish in Figs. 3 and 4, there is a remarkable difference among the numbers of nematode found in the varieties concerned. A close examination, however, indicates that no general relationships exist between the rate of larval entry and the degree of resistance, often the parasites found in the resistant variety being rather more than those in the susceptible one.

Thus the validity of the assumption, being set at the start of Experiment I, that the nematode may select its host plant has become quite untenable, and now a new question has arisen why galls are hardly formed in a resistant variety in spite of entry of the nematode.

F. Rapidity of development of the root-knot nematode in the root of different varieties

As pointed out in the case of Experiment II, the rapidity of development of the nematode is connected with the host variety. In order to reexamine this respect, some series of experiments were carried out with the varieties employed in Experiment IV.

Experiment V. A vine-cutting of one-node of each variety was respectively planted in a petri dish (10 cm in diameter) containing sand on August 5th, 1949. Three series of dishes thus treated were prepared. Inoculation was made with nematode larvae from kidney bean on August 10th in Series a, on 6th in Series b, and on 19th in Series c respectively. Plants were supplied with Pfeffer's solution ($CaNO_3$ 4 g, KNO_3 1 g, $MgSO_4$ 1 g, KH_2PO_4 1 g, KCl 0.5 g, $FeCl_3$ trace, Water 6 l). When the following intervals elapsed after inoculation respectively: 7 days in Series a, 14 days in Series b, and 21 days in Series c, nematodes within galls were examined under a microscope and distinguished in the following five developmental stages: the first-, the second-, the third-stage larva, young adult, and egg-laying female. From these data the index of development of the nematode in each variety was calculated according to the following formula.

$$\text{Index of development} = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{N},$$

where n_1, n_2, ... n_5 correspond to the number of individuals belonging to the five classes in order mentioned above, N is a total number of individuals, and 1, 2, ... 5 are the rating given to the five classes respectively. It means, therefore, that the greater the index is, the greater is the rapidity of development. The results obtained are summarized in Table 7.

A statistical examination of data shown in Table 7 reveals that there is a significant varietal difference in the index of development of the nematode at 1% level. Development of the parasite in Norin No. 3, Norin No. 9, Norin No. 2, Taihaku and Ibaraki No. 1 is noticeably retarded as compared with that of other varieties such as Gokoku, Oiran and Norin No. 1. These differences coincide for the most part with those of the known degree of resistance. Based upon experiments with pineapple and cowpea, Godfrey and Oliveira (1932) placed on record that the host plant itself, besides temperature, plays an important part in influencing the length of life history of the root-knot nematode. And they put forward a view that this might be a matter of nutrition. In the writer's case also, varietal differences in the developmental rapidity of nematode may be ascribed to the difference of nutrition which is related to the development of giant cells which are considered to act like nectaries in providing a continuous supply of nutrient mate-

Table 7.

Index of development of nematodes in different varieties of sweet potato

Variety	Ser.	No. of galls	No. of nemas	Index	Variety	Ser.	No. of galls	No. of nemas	Index
Norin No. 1	a	3	4	1.8	Kyushu No. 5	a	0	0	0
	b	2	2	3.0		b	19	32	2.7
	c	15	17	3.6		c	25	44	2.8
Norin No. 2	a	0	0	0	Kyushu No. 13	a	0	0	0
	b	4	10	1.5		b	8	11	2.4
	c	2	3	1.0		c	6	17	2.1
Norin No. 3	a	0	0	0	Kyushu No. 15	a	0	0	0
	b	0	0	0		b	16	44	1.8
	c	1	1	1.0		c	12	21	2.7
Norin No. 4	a	3	3	1.3	Tsurunashi-genji	a	3	8	1.3
	b	2	5	1.4		b	18	19	2.5
	c	10	22	2.5		c	4	5	2.0
Norin No. 5	a	1	1	2.0	Oiran	a	17	56	1.6
	b	22	28	2.5		b	11	13	3.4
	c	10	16	2.8		c	14	20	3.7
Norin No. 6	a	9	18	1.3	Okinawa No. 100	a	2	2	2.0
	b	17	22	2.5		b	20	41	2.3
	c	15	28	3.0		c	10	23	2.4
Norin No. 7	a	1	1	1.0	Ibaraki No. 1	a	5	5	1.6
	b	3	3	2.0		b	2	27	1.1
	c	1	1	2.0		c	5	6	1.0
Norin No. 8	a	7	16	1.1	Kokei No. 3	a	16	26	1.6
	b	18	35	1.9		b	12	13	2.8
	c	11	17	2.6		c	7	14	1.9
Norin No. 9	a	0	0	0	Gokoku	a	8	10	1.8
	b	1	1	1.0		b	13	25	2.6
	c	0	0	0		c	5	6	4.5
Taihaku	a	1	1	1.0	Shichifuku	a	11	12	1.3
	b	3	3	1.0		b	10	26	2.1
	c	3	3	1.3		c	22	36	2.9

rial for the continued growth of the nematode.

Up to this time the writer unfortunately had no opportunity to refer to the work of Barrons (1939) who, with reference to other crops than sweet potato, described that the root-knot resistance was not due to the lack of entry of nematode larvae, but to the interruption of the continued growth by starvation. In this connection Barrons advanced a hypothesis that certain chemicals that would inhibit the giant-cell-inducing effect of the salivary secretion of the nematode might be synthesized in the root of resistant plants. Recently Mizokami (1947), studying the microscopic sections of sweet potato rootlets infected by the nematode, reported that giant cells in highly resistant varieties were not formed at all or, if any, destroyed early in their life. The writer is of the same opinion, though he has nothing to offer in relation to the cause of non-formation of giant cells, so far as his experiments are concerned.

G. Stimulative of the root to the root-knot nematode

As already stated, the larvae of the nematode enter the root at the portion close to the tip. According to Byars' (1914) experiments with cowpea seedlings cultured in Pfeffer's nutrient agar, the larvae aggregate near the root tip. Based upon this fact, Steiner (1925) puts forward a view that the root tip possesses chemotactic stimulus which is perceived by the nematode with a sense organ called amphids which have an opening near the mouth. On the contrary, some authors (Jones, 1932, et al.) are of an opinion that the nematode attacks the loosely massed cells of the meristematic tissue of the root tip, and so gains entrance to the root.

In order to ascertain whether or not the root tip is the centre which secretes the stimulative in question, the following experiment was conducted. Adventitious roots of sweet potato treated in the following three ways were prepared: (a) the roots whose apical meristem was entirely cut off, (b) the roots whose tip was cut off leaving a part of meristem, and (c) the untreated roots. Fifteen roots, five for each treatment, were radially arranged on a watch-glass with their distal portion toward a centre of the glass, where a number of nematode larvae were liberated. Then the roots were covered with moistened, fine soil, and after 24 hours the number of nematodes which entered every root was examined. The results obtained are shown in Table 8.

Table 8.

Effect of removal of apical meristem upon entry of nematode

Treatment of root	Roots of Genji						Roots of Gokoku					
	1	2	3	4	5	Total	1	2	3	4	5	Total
Meristem entirely cut off	0	1	0	2	1	4	4	0	3	7	4	18
Meristem partially cut off	1	37	61	0	32	131	29	2	5	22	5	63
Untreated	1	47	0	1	18	67	1	28	6	20	10	65

When the meristem was partially cut off, as many or more larvae as compared with the untreated root entered the root tissue through the cut end. However, much less number of larvae were found to enter the tissue, when the meristem was entirely removed. In the latter it seems that the larvae are in all probability unable to perceive the root, owing to a faint degree of chemical stimulus, unless they come by chance in contact with the cut end. Judging from these facts, it may be stated that the apical meristem of the root is a centre from which the stimulative secretion in question is produced. As shown in Fig. 5 and observed in cowpea and pineapple by Godfrey and Oliveira (1932), the larvae often enter in a cluster at the same or adjacent point of the tip. This state of things is probably due to a large quantity of stimulative discharged through the entrance which is made by the pioneer.

Subsequently an attempt was made to compare the behavior of larvae to the root tip which was killed with boiling water with that to the living one, adopting the same method as in the preceding experiment. The results obtained are shown in Table 9.

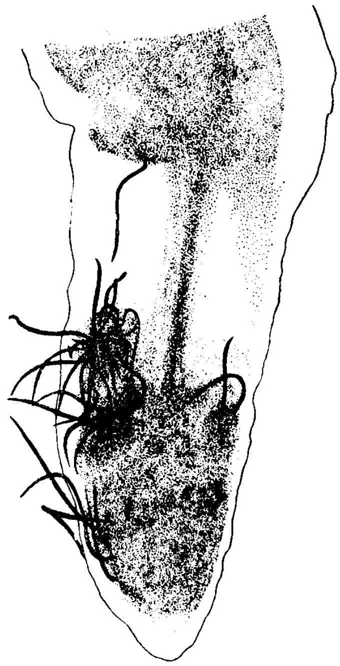

Fig. 5. Nematode larvae entering in a cluster a rootlet of "Norin No. 7".

Table 9.

Comparison of numbers of larvae which entered dead and living root tips

Treatment of root	Root No.					
	1	2	3	4	5	Total
Dead tip	64	5	57	7	2	135
Living tip	0	2	0	0	0	2

Note: The variety employed was Gokoku.

It is evident from the results that the dead tip attracts by far more strongly the larvae than the living one. The stimulus of the former, however, is probably different in chemical nature from that of the latter, because the dead part immersed in hot water becomes brown in colour and decayed owing to the effect of contaminated microorganisms.

Further experiments were made by the same method with reference to the root whose apical meristem was previously cut off and then the cut end was immersed in the boiling water, in addition to the above mentioned dead and living root tips. The number in comparison of larvae which entered the root tissues concerned is given in Table 10.

Table 10.

Comparison of numbers of larvae which entered dead and living tissues

Treatment of root	Root No.					
	1	2	3	4	5	Total
Dead cut end	8	1	1	14	16	40
Dead tip	1	0	46	3	4	54
Living tip	0	0	0	0	4	4

Note: The variety Gokoku was employed.

It is evident from the above table that the dead tissues, regardless of the presence of the meristem, stimulate and attract the larvae much more strongly than the living tissue. For the reason as stated already the stimulative of the dead tissues may be considered to be a certain decomposed product of the root tissue other than that secreted from the living root.

With regard to this respect, worthy of notice is the fact that Rensch (1924), according to Steiner (1925), confirmed that two synthetical products, the one being a known stuff from roots of many plants and the other being formed by the transformation of plant plasma in the soil, stimulated the larvae contained in cysts of *Heterodera schachtii* to hatch in the same manner as washings of roots of growing sugar beets. Steiner, however, claimed that the compounds which stimulate the larvae of *H. schachtii* to hatch are not identical with the ones which direct them afterwards to their preferred host plant; the former compounds being of little or no specific nature, while the latter of more specific nature.

As far as the root-knot nematode is concerned, the stimulative of growing roots may in all probability be common to many plants irrespective of species or varieties. Although at the present time the writer has no knowledge of the true character of the stimulative, he wishes to add here some results of observations. The apical meristematic portion of the root differs from other parts in colouration of milky-white, but it is not clear whether this difference is of physical or of chemical nature. At the root tip a biuret reaction of protein takes place, but neither fat nor starch is detected by reagents such as osmic acid, Sudan III, Nilblau and iodine-potassium-iodine.

H. Comparative susceptibility of sweet potato varieties to potassium chlorate injury and its relation to the resistance to the root-knot nematode

Since the sweet potato improvement program was commenced in 1925 at the Okinawa Agricultural Experiment Station, a number of superior varieties which gave a good yield were developed by means of cross-pollination. In breeding, however, no attention was paid to the root-knot nematode resistance, because the nemic pest was still out of consideration. So far as aware, the degrees of resistance to the root-knot nematode of parents and their hybrids stand in relation as in Table 11.

Table 11.
Relations between degrees of root-knot nematode resistance of parents and their hybrids

	Father / Mother	○						◑						●	
		Taihaku	Norin No. 2	Norin No. 5	Norin No. 7	Kyushu No. 5	Kyushu No. 6	Okinawa No. 1	Okinawa No. 100	Okinawa No. 104	Norin No. 4	Kyushu No. 8	Gokoku	Shichifuku	Norin No. 6
○	Yoshida								○◑	○					
	Taihaku												○		
	Norin No. 2										○				
	Norin No. 5								○						
	Ibaraki No. 1						◑								
◑	Okinawa No. 1								○						
	Kanto No. 3		●												
	Kyushu No. 8								○						
	Gokoku			○	○							○◑			●
●	Genki													◑●	
	Tsurunashi-genji	○		○					●						
	Shichifuku							◑		●					
	Kyushu No. 1					◑									
	Kanto No. 4						◑								

Note: ○ resistant, ◑ less resistant, ● susceptible.

Although it is not easy to draw conclusion regarding the hereditary relation because the sweet potato varieties are not only of heterozygosity but also of self-sterility, the above table seems to suggest that the root-knot nematode resistance is a heritable character which is dominant.

It is of interest to find any character which is closely associated with the resistance in question and consequently serves as an indicator to select a resistant variety. However, any trace of morphological characters in tubers, vines and leaves which are available such indicators could not be detected at all. With reference to physiological character, on the other hand, some observations were made by Poole and Schmidt (1927), showing that the susceptible varieties to stem rot (Fusarium rot) are resistant to the root-knot nematode and *vice versa*.

According to Yamasaki (1931 a) and other authorities, the resistance of certain plants to the toxic action of potassium chlorate correlates with some other physiological characters, such as cold hardiness, drought resistance, hygienic condition, and sex of dioecious plants. These results raise the question whether there are differences of resistance to the chlorate among varieties of sweet potato, and, if so, what relationship exists between the resistances to the toxicant and to the nematode. In order to solve this question the writer made a series of experiments in 1946–1949.

On Sept. 5th, 1946, distal portions of vines, about 1 ft. long, of two varieties of sweet potato, Norin No. 2 (resistant to the nematode) and Genji (susceptible to the nematode), were cut from a field and cultured in various concentrations of potassium chlorate (0.05, 0.1, 0.15 and 0.2%) for various hours (6, 24 and 48 hours) under the laboratory conditions. The degree of resistance to the toxicant was determined by the degree of symptom of chlorate injury and the time needed up to its appearance. The symptoms appear in sweet potato as follows: a chlorosis of leaf that results in the appearance of a mottling or a uniform pale green colour; brown spots in the chlorotic area; wilting, rolling up and necrosis of an entire leaf; scald-like spots or stripes on vine.

As a result of experiments it may be stated that there is a remarkable difference in resistance to the toxicant between the two varieties concerned. Norin No. 2 is much more susceptible than Genji. This seems to suggest that the nematode-resistant variety is susceptible to potassium chlorate and *vice versa*.

Similar experiments were repeated under the combination of the concentration of 0.2% $KClO_3$ and the cultural period of 24 hours, employing fourteen varieties on Sept. 11th, 1946 and thirty-eight ones on August 18th, 1947. The results obtained are summarized in Tables 12 and 13.

Table 12.

Relation between the resistances to the nematode and to the chlorate (1)····1946.

Susceptibility to chlorate	Resistance to nematode		
	Susceptible var.	Intermediate var.	Resistant var.
Susceptible varieties		Okinawa No. 100	Norin No. 2 Norin No. 3 Norin No. 7 Kyushu No. 8
Intermediate varieties	Shichifuku Tsurunashi-genji Hayato	Gokoku	Kyushu No. 3
Tolerant varieties	Norin No. 1 Oiran Genji	Kokei No. 3	

Table 13.

Relation between the resistances to the nematode and to the chlorate (2)····1947.

Susceptibility to chlorate	Resistance to nematode		
	Susceptible var.	Intermediate var.	Resistant var.
Susceptible varieties	Kyushu No. 12 Kanto No. 21 Kanto No. 3 Kanto No. 19 Norin No. 6	Kanto No. 22 Okinawa No. 100 Norin No. 4	Ibaraki No. 1 Norin No. 5 Norin No. 2 Kanto No. 24 Norin No. 10 Kyushu No. 3
Intermediate varieties	Kagoshima Shichifuku Kanto No. 5 Kyushu No. 1 Norin No. 1 Tsurunashi-genji Hayato	Kokei No. 3 Norin No. 8	Kanto No. 23 Kyushu No. 4 Kyushu No. 6 Norin No. 3 Kyushu No. 5 Norin No. 7
Tolerant varieties	Kyushu No. 10 Genji Oiran	Kanto No. 25 Gokoku	Taihaku Kyushu No. 11 Kyushu No. 8 Kanto No. 18

In comparison of the data in 1946 and 1947 it is, contrary to expectation, revealed that the reaction which the same sweet potato variety shows to the toxicity of $KClO_3$ is not necessarily constant but apt to vary under different conditions. For example, Norin No. 3, Norin No. 7 and Kyushu No. 8 which were ranked in susceptible group in 1946 exhibited a tendency toward much tolerance in 1947, and, on the contrary, Norin No. 1 and Kokei No. 3 were definitely less tolerant in 1947 than in 1946. The data in 1947, moreover, reveal the fact that some varieties are resistant to both the nematode and toxicant, while some are susceptible to these two.

Table 14.
Susceptibility to chlorate injury of individual leaves cultured in 0.2% $KClO_3$ for 24 hours

Varieties	Series A			Series B			Series C			Total number of leaves		
	Leaf No.			Leaf No.			Leaf No.					
	1	2	3	1	2	3	1	2	3	○	◑	●
Norin No. 1	○	◑	●	○	●	◑	◑	●	●	2	3	4
〃 No. 2	◑	●	●	●	◑	●	●	●	●		2	7
〃 No. 3	◑	○	○	○	◑	◑	○	◑	◑	4	5	
〃 No. 4	◑	◑	●	●	●	●	●	●	●		2	7
〃 No. 5	◑	○	●	●	●	●	○	◑	●	2	2	5
〃 No. 6	◑	●	●	◑	◑	◑	●	○	◑	1	5	3
〃 No. 7	◑	○	●	○	◑	○	○	○	○	6	2	1
〃 No. 8	○	○	○	●	●	●	○	○	○	6		3
〃 No. 9	◑	◑	●	●	◑	●	◑	●	○	1	4	4
Okinawa No. 100	●	●	◑	●	●	◑	●	●	●		2	7
Gokoku	○	◑	○	○	○	○	○	○	○	8	1	
Taihaku	○	○	○	○	○	○	●	○	◑	7	1	1
Tsurunashi-genji	○	○	●	○	◑	◑	○	◑	◑	4	4	1
Oiran	○	○	○	◑	◑	◑	○	○	◑	5	4	
Shichifuku	○	○	○	●	●	○	◑	●	●	4	1	4
Kokei No. 3	○	○	○	●	◑	●	◑	●	●	3	2	4
Ibaraki No. 1	●	◑	●	●	◑	○	◑	◑	●	1	4	4
Kyushu No. 5	○	◑	◑	◑	●	◑	◑	◑	◑	1	7	1
〃 No. 13	◑	◑	○	○	○	◑	◑	◑	◑	3	6	
〃 No. 15	◑	◑	◑	○	○	◑	◑	◑	●	2	6	1

Note: ○ tolerant, ◑ intermediate, ● susceptible

According to Yamasaki (1931 b), toxic action of $KClO_3$ depends on the toxic ion, ClO, which is reduced from $KClO_3$ in the presence of such substances as glucose, aldehydes, etc. It is therefore suggested that the flactuation of susceptibility to the chlorate injury of the same variety is due to the difference of the amount of reducing substances accumulated by the assimilation, the intensity of which varies under different conditions. That even leaves of the same variety which were picked from a field at the same time did not always show the same symptom would be confirmed from the experimental results in 1949 given in Table 14.

In conclusion, the resistance to the root-knot nematode of the sweet potato variety is not capable of judging from the reaction to the chlorate injury.

V. Conclusion

Larvae of the root-knot nematode are attracted by a certain chemotropismic stimulative which is secreted from the apical meristem of the growing root of plants. This stimulative is regarded to be in all probability of a nature common to several kinds of plants, but not peculiar to any plant itself. Accordingly the nematode is capable of perceiving any root of plants, irrespective of species or varieties, without undergoing host selection at all. The larvae thus attracted arrive at and enter the portion close to the apical meristem. When the conditions within the root are suitable, the further growth of the larvae goes on after successful entrance. It seems more reasonable to consider that the conditions which affect the establishment of the nematode are attributed to certain chemical or physiological characteristics of the root rather than morphological ones. The ability of the nematode to thrive after entrance may be dependent on the possibility of the formation of giant cells which are induced by the stimulation of the nematode itself and moreover serve as a source of its nutrition. In the resistant host plant giant cells are not altogether developed on account of a certain reason. As a result the nematode comes to die sooner or later from hunger. As to the reason in question Barrons (1939) put forward, as already noted, a view that the resistant host is able to synthesize certain chemicals which counteract the giant-cell-inducing effect of the saliva of the nematode. Although the writer has at present no knowledge of the nature of such hypothetical substances, it is believed that the most hopeful line of attack in the determination of the cause of giant-cell-formation is an examination of the proteins of resistant and susceptible varieties by immuno-chemical methods. The proteins from the resistant variety probably possess a serological peculiarity which is absent from these extract from the susceptible one.

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