STUDIES ON WITCHES' BROOM OF SOYBEAN[1]

TSING-CHE LO and YOU-HSIN HAN[2]

(Received Dec. 10, 1968)

Introduction

Soybean witches' broom, first reported by Han (1959), has become widely distributed in Taiwan in the recent years. The diseased plant usually fail to produce pods and, hence, are given the name "male beans" by the local soybean growers. In the central and southern parts of the island where a rapid increase in the soybean acreage has occurred, the disease reduces the yield considerably and heavy losses in soybean production result. Usually, the spring and summer crops sustain greater losses than the winter crop.

Because the disease produces symptoms which resemble the witches' broom diseases incited in many other crops by viruses (some of them that were originally described as virus are now considered as pleuropneumonia-like organisms or mycoplasma), a vector is suspected to mediate the disease spread in the field. Attempts were, therefore, made to clarify the transmission method and vector of the disease, and based on the production of symptoms produced in transmission experiments, the disease was further characterized. The results of these attempts are presented in this paper.

Materials and Methods

The diseased plants employed in transmission experiments were collected from Hsinchu, Taichung and Pingtung. Soybean plants with typical witches' broom symptoms were grown in pots in the greenhouse, they were used as sources of inoculum. The soybean variety Palmetto was used as test plants throughout the experiment. Seeds of soybean were sown in steam-sterilized soil and seedlings were thinned to 1 per pot. In 2 weeks when the seedlings had reached the 3 leaf stage, they were inoculated. In mechanical transmission experiments, extracted juice from the diseased plants was rubbed on leaves of healthy seedlings with the aid of carborundum. Seeds used for

---

1. Grateful acknowledgment is made to Dr. H. Hasegawa for identifying test insects. Particular thanks are extened to Prof. J. J. Chiu for reading of the manuscript, and to Mr. T. S. Tsai for helping experimented works.

2. Professor (deceased) and Associate Professor, Department of Plant Pathology, Taiwan Provincial Chung Hsing University.

testing seed transmission were collected from diseased plants in a heavily infected field plot. To determine the possibility of disease transmission by an insect vector, various insects were collected from the soybean as well as the peanut fields. They fed separately on infected plants before being transferred to healthy plants for inoculation feeding. Susceptibility of various plants to the disease was tested after the vector insect was found.

Results

Symptoms on soybean

1. External Symptoms: According to field observations, the characteristic symptoms of the disease are phyllody of the blossoms, small leaves, and the abnormal excessive development of buds leading to a bushy broomy appearance. Small leaves are formed as a result of antholysis. Since soybean plants can be infected at different times from the seedling stage to maturity, the symptoms are often variable. When plants are infected in their early stage, floral abnormalities predominate. These abnormalities madifest as phylloid transformation of sepals and calyxes into green color leaflets. Ovaries are often deformed as small leaf-like organs. There are many small branches which bear aboundant small leaves on nodes. This is especially conspicuous in the tall varieties such as Palmetto, Pelican, Acadian, and Taiwan green bean, etc. as well as when the planting is thick.

The flowers of the diseased plants are larger than the healthy ones. When the flower is not completely deformed, some flower parts are differentiated in that the sepals and petals change into leaflets either with or without short petioles. In most cases the pistil may also change into a green color leaflet, but sometimes, the normal pod form still remains and the suture of the pod is not completely closed at its free end. The leaflets thus formed are so irregular in their form that they stand erect or bend slightly. Usually, the ovules disappear. Stamens of the infected plants are the only parts that are not phylloid. However, they are changed from diadelphous forms into polydelphous, with stamens completely separate. The filaments of the stamens are short, the anthers are small and usually no pollen grains can be found. Since all the flower parts are deformed into leaf-like structures and abnormal branching also occurs, this results in the production of many small leaves and luxuriantly growing weak branches.

Symptoms of soybean witches' broom are quite similar to those of the witches' broom of leguminous plants (Murayama 1966, Shinkai 1964). If infection takes place later than that mentioned before, usually a lesser degree of phyllody may appear in some flowers while most still maintain their normal features. Abnormal branches and swollen nodes are found only occasionally.

Pods of normal or smaller size can be produced under such conditions. One or two small seeds may be found within the pod. If infection takes place in an even later stage healthy pods are produced, although many adventitious buds may be formed. The majority of these adventitious buds fail to extend. Finally, vein yellowing may be seen in old leaves of infected plants. It is evident that the gradation of blossom phyllody, number of abnormal branches, and possibility of pod formation are correlated to the time of infection. In the fields, the plants infected by this disease live longer than healthy ones. Although the disease is systemic in nature, some of the infected plants may produce one or more branches without symptoms.

2. Internal Symptoms: To understand histological and cytological changes of the infected plants, freehand sections through the stems, branches and petioles of both diseased and healthy plants of the variety Palmetto from the greenhouse were examined under the light microscope. Fresh pieces of tissue were stained with Giemsa solution according to Bald (1949) as modified by Rawlings (1957) for observation of intracellular inclusion bodies. Iodine solution was also used to stain the cut tissues for the observation of carbohydrates. Microscopic examinations of the leaf tissue revealed no X-bodies in sections of the diseased plants. But it should be pointed out that excessive accumulation of carbohydrates, particularly of starch grains, has been found to occur in stems, branches and petioles as a consequence of infection. The starch grains, in general, were round and were more numerous and larger than in healthy ones. Mostly they were found in the parenchymatous cells of vascular tissue and occasionally in pith. Histological examination revealed greatly thickened in the vascular bundles, especially from the abnormal branches.

Transmission experiments

1. Mechanical transmission: Inoculum was prepared by grinding leaves of infected plants or the phylloid flower parts in an equal weight of a buffer solution (0.05M K_2HPO_4, with pH adjusted to 7.2). The juice was pressed through a double-layer fine cheesecloth. Soybean varieties Lincoln, NTUKS No. 1, and Palmetto, the tobacco variety Vanheik and black-eyed cowpea were used as the assay plants in this test. Since shading or complete darkening before inoculation has been shown to increase the susceptibility of some assay plants to viruses (Bawden and Roberts 1948), the test plants were placed in the dark room for one day before inoculation. Leaves were first dusted with 400-mesh carborundum and virus was applied with cheesecloth. The inoculated leaves were washed immediately with tap water after inoculation. The plants were then placed in the greenhouse. Several tests were made at different times of the year. Of 280 inoculated plants of the soybean variety Lincoln, 230 plants

of the variety Palmetto, 100 cowpea plants and 90 tobacco plants (variety Venheks), none of them showed symptoms. Thus, it is concluded that the disease is not transmitted mechanically from plant to plant.

2. Seed transmission: Soybean seeds of the variety Palmetto were collected from infected plants from Likong (里港) and Pingtung (屏東). All seeds were sown in sterilized soil in 8 in. pots which were placed in a screen-house free from insects. Experiments made at different times showed a lower germination percentages of seeds collected from diseased plants than those from healthy plants. All plants were normal and no symptoms appeared. These results Table 1) give no indication that the disease was transmitted through seeds.

Table 1. *Results of seed transmission experiments on soybean witches' broom in the variety Palmetto*

Experiment	No. of seed sowed	No. of plants germinated	No. of plants showing symptoms .
1	60	47	0
2	90	74	0
3	1C0	83	0

3. Insect transmission

To determine whether soybean witches' broom can be transmitted by insects, various species of insects were collected from fields where soybean and penaut were grown. Those which did not survive on soybean plants were tested immediately after collection, while the following species were tested either shortly after collection or by using their offspring produced in the feeding cages: *Aphis glycines* Matsumura, *Chlorita formosana* Paoli, *Nesophrosyne orientalis* Matsumura, *Hishimonas disciguttus* Walker, and *Strogania diminuta* Matsumura. In tests with *A. glycines*, the aphids were allowed an acquisition feeding for 60 minutes and then carefully transferred to healthy plants with a chinese brush. Inoculation was done less frequently by placing on the test plants a detached diseased leaf of soybean on which aphids had been feeding and from which the aphids moved to the healthy plants when the diseased leaf wilted and dried. At the end of seven days all plants were sprayed with insecticides to kill all insects and were moved to a greenhouse section free from insects for periodic observations. None of 3240 individuals of the aphid tested in 3 experiments transmitted this disease, indicating that soybean witches' broom is not transmitted by *A. glycines*. Insects other than the aphids were tested with various periods of acquisition feeding and inoculation feeding. Data presented in Table 2 revealed that soybean witches' broom was specifically transmitted by *Nesophrosyne orientalis* but not by any other insects that were tested.

Table 2. *Preliminary screening of insects for transmission of soybean witches' broom.*

Insects	Acquisition feeding period (days)	Inoculation feeding period (days)	Transmission efficiency
Chlorita formosana Paoli	7-21	7-20	0/118[1]
Euscelis obscurinervis (Stal.)	—[2]	2	0/2
Cicadella sexnotata Fall	—	5-6	0/10
Chlorita biguttula Ishida	—	3-6	0/90
Scaphoideus festivus Matsumura	—	6	0/2
Nezara antennata Scott	—	7-9	0/34
Cicadella spectra Distant	—	6	0/2
Nirvana palida Melich	—	6-10	0/8
Hishimonus disciguttus Walker	—	1-10	0/14
Ditto	30	8-10	0/8
Strongania diminuta Matsumura	—	7-10	0/12
Ditto	10-32	7-11	0/13
Nesophrosyne orientalis Matsumura	—	12	2/2
Ditto	—	6	0/2
Ditto	—	7	2/2

(1) The numerator is the number of plants that became infected, and denominator is the number of seedlings that were infested with the test insects.
(2) Insects were collected from the fields; no acquisition feeding was allowed in the greenhouse.

4. Transmission of soybean witches' broom by *Nesophrosyne orientalis*

(1) Efficiency of Transmission

In order to provide further evidence that soybean witches' broom can indeed be transmitted by *N. orientalis*, experiments were carried out using cultures of this insect established from small nymphs which had no previous access to diseased plants and which were believed to be free from infection with the disease inciting agent. Leafhoppers from these healthy cultures were allowed two weeks or longer for acquisition feeding before they were trans-

Plate I

1-4. Soybean infected with witches' broom. 1-3. Abnormal branches, 4. Phylloid blossom.
5. Phylloid blossoms of kidney bean infected with soybean witches' broom.
6. Two kidney bean plants of same age: plant on the left was infected with soybean witches' broom; plant on the right was healthy.
7. Cowpea infected with soybean witches' broom showing numerous upright-growing branches.
8. Peanut infected with soybean witches' broom showing abnormal clusters of branches.
9. Pea infected with soybean witches' broom showing the abnormal branches.
10. Three globe amaranth plants of same age. Two plants on the left were healthy; plant on the right with abnormal branches was infected with soybean witches' broom.


1
2
3
4
5
6
7
8
9
10


11
12
13
14
15

ferred to the test plants where they remained for 3 to 10 days. In Table 3 it is seen that 87 of the 119 soybean plants that were inoculated from the leafhoppers were infected. The results strongly indicate that *N. orientalis* is an efficient vector of witches' broom.

(2) Incubation period in the vector

Healthy leafhoppers were caged on diseased plants for acquisition feeding for 24 or 48 hours. They were then individually transferred to healthy seedlings. Successive transfers to a new plant were made at 24– or 47–hour intervals, depending on the experiments, until the insects died or the test was ended. All plants thus inoculated were placed in the greenhouse for daily observation.

Table 3. *Transmission of soybean witches' broom by* ***Nesophrosyne. orientalis*** *under the greenhouse conditions.*

Acquisition feeding period (days)	Transmission feeding period (days)	No. of leafhoppers used and surviving[2]	Successful transmission[3]
14	3	3/3	4/4
14[1]	5	22/28	24/35
14[1]	6	15/21	26/36
14[1]	7	26/30	18/25
14	8	12/17	11/16
14	10	1/3	4/4

(1) More than 14 days.
(2) Numerator is the number of leafhoppers survived at the end of transmission feeding. Denominator is the number of leafhoppers used in transmission feeding.
(3) Numerator is the number of plants that became infected, denominator is the number of seedlings that were infested with leafhoppers.

Plate II

11. An abnormal branch of radish plant infected with soybean witches' broom. Note the phylloid blossoms.

11–13. Sun hemp plants: 12. two plants on the left were infected with soybean witches' broom, showing abnormal branches with antholysis and phylloidy of blossoms; two plants on the right were healthy. 13. Floral abnormality of sun hemp plant infected with soybean witches' broom.

14. Two crotalaria plants of same age: plant on the left was infected with soybean witches' broom showing abnormal branches; plant on the right was healthy.

15. Zinnia infected with soybean witchs' broom showing the abnormal branches deformed from floral parts and phylloid blossoms.

16. Two "Fukwei" pea plants of same age: Plant on the left was healthy; plant on the right was infected with soybean witches' broom, showing abnormal branches.

17. Snapdragon infected with soybean witches' broom, showing abnormal branches with antholysis and phyllody of blossoms.

18. Common cosmos infected with soybean witches' broom showing the abnormal branches.

19. Hairy indigo infected with soybean witches' broom showing the abnormal branches.

The experimental data summarized in Table 4 indicate that a minimum of 13 days after starting the acquisition feeding was required for the insects to transmit the disease successfully. Many individuals of the leafhopper failed to transmit. This could be explained that either the leafhoppers did not acquire the pathogen or the prospective vector leafhoppers died before they were able to transmit the disease.

Table 4. *The latent period of soybean witches' broom pathogen in N.* ***orientalis.***

Acquisition feeding period (hrs)	Identity of the test insects															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
24 hrs. Days the test insect survived	12	10	31	25	12	11	22	23	10	16	33	33	12	9	—	—
Latent period (days)	—[1]	—	21	17	—	—	—	—	—	—	—	19	—	—	—	—
48 hrs. Days the test insect survived	8	18	5	4	26	7	26	18	5	10	29	17	45	31	24	7
Latent period	—	—	—	—	—	—	—	16	—	—	13	—	—	20	16	—

(1) No symptoms (infection) appeared on soybean variety Palmetto.

5. Tests on susceptibility of various plants

The following plants were tested in transmission experiments for their susceptibility to the disease using infected leafhoppers as vector: peanut (*Arachis hypogaea* L.), cowpea (*Vigna sinensis* L.), kidney bean (*Phaseolus vulgaris* L.), zinnia (*Zinnia elegans* L.). sun hemp (*Crotalaria juncea* L.), common cosmos (*Cosmos bipinnatus* Cav.), radish (*Raphanus sativus* L.), pea (*Pisum sativus* L.), globe-amaranth (*Gomphrena globosa* L.), broad bean (*Vicia faba* L.), cornflower (*Centaurea cyanus* L.), snapdragon (*Antirrhinum majus* L.), red bean (*Phaseolus radiatus* L. var. *aurea* Prain), "Fukewi" pea (*Mucuna capitata* W. et A.), crotalaria (Crotalaria *saltiana* Andr.), centro (*Centrosema pubescens* Benth), spanich needles (*Bidens bipinnata* Linn.), tropic ageratum (*Ageratum conyzoides* Mill), lambsquarters goosefoot (*Chenopodium album* Linn.), asian pigeonwings (*Clitoria ternatea* L.), rhodesian kudzu (*Glycine javanica* L.), and hairy indigo (*Indigofera hirsuta* Linn.). The results are presented in Table 5. The infected plants, in general, showed symptoms of phyllody of the blossoms, and an abnormal, excessive and premature development of adventitious buds which led to extravagant branching. As a result the plants developed a bushy, broomy appearance. Contrary to the usually abnormally large phylloid branches in other infected plants, the blossoms of globe-amaranth were smaller than normal, although the change from light to dark green color in petals is quite common. The observed incubation period of the disease varied with different species of the plants tested. Thus, cowpea, sun hemp, and kidney bean showed witches' broom symptoms earlier than any other plants tested, while broad bean had the longest incubation period.

Table 5. *Tests on susceptibility of various plants to soybean witches' broom as transmitted by N. orientalis in the greenhouse.*

Test plant	No. of plants inoculated	No. of plants infected	Percentage of infection	Incubation period of the disease(days)
Arachis hypogaea L.	40	27	67.5	43-64
Vigna sinensis L.	45	28	62.2	19-34
Phasiolus vulgaris L.	29	26	70.1	16-23
Zinnia elegans jacq.	24	7	25.0	34-41
Crotalaria juncea L.	77	57	74.0	22-33
Crotalaria saltiana Andr.	5	4	80.0	26-31
Cosmos bipinnatus Cav.	48	14	29.2	37-49
Raphanus sativus L.	31	17	54.8	33-49
Pisum sativus L.	25	17	68.0	29-36
Gomphrena globosa L.	53	9	16.9	35-67
Vicia faba L.	15	9	60.0	60-63
Antirrhinum majus L.	27	5	71.4	46-56
Centaurea cyanus L.	4	4	100.0	24-32
Mucuna capitata W. et A.	5	5	100.0	45-46
Phaseolus radiatus L. var. *aurea* Prain	3	2	66.6	33-26
Centrosema pubescens Benth	11	4	36.2	40-50
Glycine javanica L.	5	3	60.0	25-30
Indigofera hirsuta Linn.	5	3	60.0	
Bidens bipinnata Linn.	9	1	0	
Ageratum conyzoides Mill	7	—(1)	0	
Chenopodium album Linn.	11	—	0	
Helianthus annus L.	44	—	0	
Callistiphus chinensis Nees.	13	—	0	
Clitoria ternatea L.	9	—	0	

(1) No symptom was observed.

Discussion and Conclusions

The experimental results obtained in this study provide evidence beyond any doubt that the leafhopper *N. orientalis* transmits soybean witches' broom. Shinkai (1964) and Murayama (1966) reported that witches' broom of leguminous plants found in the Ryukyu islands was also transmitted by this leafhopper. The witches' broom symptms consisting of small leaflets and phyllody of blossoms are some what similar to those of soybean bud blight. However, Allington (1946) and Desjardins *et al.* (1954) reported that bud blight of soybean is caused by tobacco ring spot virus (TRSV). It is transmitted through seed of the soybean variety Lincoln, and a low temperature favor the disease development. Later, Kahn and Laterell (1955) confirmed that bud blight of soybean could be transmitted by mechanical means. From this point of view, the witches' broom of soybean described in this paper is different from bud blight.

Until very recently, a plant disease was assumed to be of virus nature if its transmission is mediated by a leafhopper and if attempts to culture its causal agent fail. Aster yellows and a number of plant diseases producing phyllody symptoms are well known examples. However, recent evidence obtained from electron microscopic studies by Doi *et al.* (1967), Maramorosch *et al.* (1968), and Ploaie *et al.* (1968) strongly suggests that several diseases of the aster yellows group is very probably not caused by a virus. In thin sections of phloem tissues prepared from plants infected with these diseases, a kind of the pleuropneumonia-like organisms (PPLO) was detected. In no case was such organism detectable in sections similarly prepared from plants which were not infected with these diseases. These findings and the fact that certain chemical compounds effective against PPLO cured the diseases when applied to infected plants led these investigators to believe that mulberry dwarf, aster yellows and related disease are probably caused by PPLO.

Whether soybean witches' broom is caused by virus or a kind of pleuropneumonia-like organisms remains to be determined. Evidences obtained in this study that the disease was transmitted by the leafhopper *N. orientalis* but not by other means, and that it produced phyllody symptoms in nearly all test plants which were found susceptible, suggest that the disease belongs to the aster yellows group. Indeed, Shikata *et al.* (1968) has found a close association of PPLO and a white leaf of sugar cane occurring in Taiwan. Work to determine the true nature of the agent inciting soybean witches' broom in Taiwan is now in progress.

Summary

Witches' broom a new diseaase of soybean found in Taiwan, is here described. The most conspicuous symptoms are phyllody of blossoms, and the development of a great number or of small leaves and abnormal branches. In most cases, no pods are produced by the infected plants, thus the soybean yield is decreased. Accumulation of starch has been found to occur in stems as a consequence of infection. The starch content is higher in pith and parenchymatous cells in the infected tissues, than in noninfected ones. Cross sections of stems and petioles of the infected plants show thickening of xylem in the vascular bundles. No inclusion bodies found in tissues of the infected plants.

The disease is not transmitted by mechanical means, nor by seed. Results of transmission experiments conducted under greenhouse conditions prove that *N. orientalis* is an efficient vector of the witches' broom pathogen. The insect infected the test plants in 18 to 21 days and 16 to 20 days following acquisition feeding for 24 hrs. and 48 hrs. respectively. The following plants were found with witches' broom symptoms after an inoculation feeding by *N. orientalis* peanut, cowpea, kidney bean, zinnia, crotalaria, common cosmos, radish, pea, sun hemp, cornflower, red bean, "Fukwei" pea, centro, rhodesian kudzu, hairy indigo, globe amaranth, broad bean, and snapdragon. Most of the diseased plants developed phyllody, certain leaflike special features and excessive abnormal branches. Typical symptoms of witches' broom can also be found on several crop plants naturally infected in the fields.

大豆天狗巢病之研究

羅清澤　韓又新

本文乃報導臺灣大豆天狗巢病之傳播試驗結果，大豆天狗巢病最顯著之病徵爲花器葉化(Phyllody) 及叢生不定枝。因罹病豆株常不結莢，影響產量至鉅。病組織顯微鏡檢查未發現封入體，莖及葉之細胞內之澱粉粒較健株者爲多。本病非種子傳播，亦非機械傳播。各種昆蟲媒介傳播試驗結果顯示南斑浮塵子 *Nesophrosyne orientalis* 爲大豆天狗巢病之媒介昆蟲。該浮塵子於病株上飼育24及48小時，再經歷潛伏期 18–21 天（爲飼育於病株上24小時，或16–20天（爲飼育於病株上48小時)，即能有效的將天狗巢病原體傳染於健全植物。接種飼育爲 3—10 天。病原體經該浮塵子傳播可侵害多種經濟植物，包括花生、豇豆、菜豆、太陽麻、波斯菊、蘿卜、豌豆、蠶豆、金魚草、紅豆、山珠兒、千日紅、百日草、野生大豆等。其疾病徵潛伏期因植物種類而異，各種植物之病徵與大豆者極其相似，亦與田間自然發生者相同。

Literature Cited

ALLINGTON, W. B. Bud blight of soybean caused by the tobacco ringspot virus. Phytopathology. **36**: 319-322, 1946.

BALD, J. G. A method for the selective staining of viruses in infected plant tissues. Phytopathology. **39**: 395-402, 1949.

BAWDEN, F. C., and RODERTS, F. M. Photosynthesis and predisposition of plants to infection with certain viruses. Ann. Appl. Biol. **35**: 418, 1948.

DESJARDINS, P. R., R. L. LATTERELL, and J. E. MITDHELL. Seed transmission of tobacco-ringspot virus in Lincoln variety of soybean. Phytopathology. **44**: 86, 1954.

DOI, Y., M. TERANAKA, K. YORA and H. ASUYAMA. Mycoplasma or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. Ann. Phytopathology. Soc. Japan **33**: 259-266, 1967.

Han, Y. H. Soybean diseases in Taiwan. Jour. Agr. Assoc. China. New Series **26**: 31-38, 1959.

KAHN, R. P., and F. M. LATTERELL. Symptoms of bud-blight of soybeans caused by the tobacco-and tomato-ringspot viruses. Phytopathology. **45**: 500-502, 1955.

MARAMOROSCH, K., E. SHIKATA, and R. R. GRANADOS. Mycoplasma-like bodies in leafhoppers and diseases plants. (Abstract), Phytopathology. **58**: 886, 1968.

MURAYAMA, D. On the witches' broom diseases of sweet potato and leguminous plants in the Ryukyu Islands. Memoirs of the Faculty of Agriculture, Hokkaido University. Vol. 6, No. 1, pp. 81-108, 1966.

PLOAIE, P., R. G. GRANADCS, K. MARAMOROSOH. Mycoplasma-like structures in periwinkle plants with crimean yellow, European clover dwarf, stolbur. (Abstract). Phytopathology. **58**: 1063, 1968.

RAWLINS T. E. A modification of Bald's stain for viruses and for cell inclusions associated with virus infections. Phytopathology. **47**: 307, 1957.

SHIKATA, E., K. MARAMOROSCH, C. C. LIN, and T. MATSUMOTO. Mycoplasma or PLT Group-like micro-organisms found in phloem elements of plants infected with yellow dwarf, corn stunt in U. S. A., yellow dwarf of rice in Phellipine, and white leaf of sugar cane in Taiwan. (Abstract in Japanese). Ann. Phytopathology. Soc. Japan **34**: 208, 1968,

SHINKAI, A. Studies on insect transmisson of sweet potato witches' broom disease in the Ryukyu Islands. Social Research Report, Agriculture Section, Economic Department, Government of the Ryukyu Island 1-45, 1964.