It is significant also that the disease usually makes its appearance in late summer and early autumn, apparently at the period when there is an acceleration of food accumulation in the bark, and develops with great rapidity after the first symptoms become visible. It has been observed also that the trees most severely affected often have the most vigorous root systems.

In view of these experimental observations a much more extensive experiment using Soil A and Soil B was set up in the greenhouse on 4 April 1945. Fifty-four trees (32 treated; 22 controls) were planted in Soil A. Ninety trees (48 treated; 42 controls) were planted in Soil B. Greater concentrations of iron and of manganese were used (96–1,536 ppm) than in the previous experiment. At all times ideal moisture conditions were provided and as a result the trees made a much more vigorous growth than did those in the previous experiment, which were watered only intermittently so as to approximate orchard conditions.

By the end of the first growing season (1945) 75 per cent of the treated trees and 45.4 per cent of the controls in Soil A were diseased. In Soil B 31 per cent of the treated trees were diseased, and none of the controls was diseased.

From the results so far obtained it is quite apparent that excess manganese and perhaps iron are important factors in the development of the disease, but in order to evaluate the specific effect of manganese, iron, or combinations of manganese and iron, further experiments must be carried out, with trees grown in pure sand culture.

On 21 April 1945 such a preliminary experiment was begun. Red Delicious apple whips cut back to 10 inches were grown in acid-washed sand with the addition of varying amounts of manganese. The basic nutrient solution used was one which the authors have found well suited for the growth of apple trees.[2] For the first three weeks after the trees were planted all trees were supplied with the basic nutrients. Throughout the experiment the control trees received this nutrient solution. The manganese added to the treated trees was increased by doubling the concentrations in geometric progression from 0.5 to 128 ppm.

Within seven days after the manganese treatments were begun the new leaves of the trees began to manifest symptoms of manganese toxicity varying in intensity with the concentration used. At the end of the first growing season the disease developed on all four trees receiving the two highest concentrations of manganese, namely 64 and 128 ppm. In this experiment the disease was unusually severe and occurred both on the old growth and on that of the current season. It is noteworthy that the time of development of the disease in sand culture coincided with the appearance of the disease in other trees grown in crocks in Soil A, whether in the greenhouse or outside, and also with the time of its development in the orchard from which Soil A was taken.

During the last five years the writers have used lime in some field experiments. In April 1945 a greenhouse experiment was begun using Soil A, the soil in which some internal bark necrosis develops. Lime was added to eight crocks of this soil at planting time to decrease the acidity from pH 4.2 to pH 6.5. Of the eight trees grown, none developed the disease. Twenty-two control trees were grown in the same soil without addition of lime, 10 of these developing internal bark necrosis.

The above-described experiments indicate that manganese and perhaps iron are important factors in the development of internal bark necrosis. Further studies, however, are necessary in order to determine what specific role they play in causing this physiological disturbance.

[2] Basic nutrient solution: dihydrogen potassium phosphate, 0.0108 M; calcium nitrate, 0.0078 M; magnesium sulfate, 0.0020 M; ammonium sulfate, 0.0007 M; boron (as boric acid), 2.0 ppm; Fe (as iron tartrate), 6.0 ppm; Zn (as zinc sulfate), 0.2 ppm; Cu (as copper sulfate), 0.1 ppm; and Mn (as manganous sulfate), 0.5 ppm. Nutrients were renewed weekly. Nutrient solution was forced upward twice daily through the sand, allowed to stand 10 minutes in contact with the sand, and then allowed to drain. The trees were grown during the entire period in a greenhouse under whitewashed glass.

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Two New Effective Insect Repellents, NMRI-201 and NMRI-448[1]

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A survey of mosquito repellents at the Naval Medical Research Institute revealed two chemicals, 2-phenyl cyclohexanol and β-tetralol (2-naphthol,1,2,3,4-tetrahydro), which were unique because they lost only a relatively small percentage of their activity when applied to the sweating skin of test subjects. A mixture of these chemicals (NMRI-201), consisting of 70 per cent 2-phenyl cyclohexanol and 30 per cent β-tetralol (by volume), was found more effective than either of

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[2] The opinions and statements are those of the authors and not necessarily those of the Navy Department.

the ingredients. Other proportions of these compounds were not as effective as NMRI-201 (Table 1).

During storage tests both tetralol and NMRI-201 developed an orange-red color. Tests against mosquitoes showed that these materials had lost some of their repellent properties. Chemical analysis indicated that oxidation had occurred. In order better to understand these changes, freshly distilled β-tetralol was oxidized in Warburg vessels for varying intervals of time and the repellent activity and color changes of each sample determined. Some correlation between extent of oxidation, amount of chromogen, and repellent activity was noted (Fig. 1).

TABLE 1

Summary of Repellency Tests Against Mosquitoes (*Aëdes aegypti*) to Determine the Most Effective Combination of 2-Phenyl Cyclohexanol and β-Tetralol

NMRI No.	Ingredients (% by vol.)		Average period of complete protection (min.)	
	β-tetralol	2-phenyl cyclohexanol	Subjects with dry skin*	Subjects with sweating skin†
164	100	0	361	143
203	70	30	303	115
202	50	50	370	122
201	30	70	451	178
192	0	100	324	157

* Environmental temperatures: 80° F. dry bulb, 70° F. wet bulb.
† Environmental temperatures: 90° F. dry bulb, 80° F. wet bulb.

Since the formation of hydroperoxides appeared to accompany the oxidative changes, β-tetralol with known hydroperoxide content was prepared, varying concentrations being tested against mosquitoes. Again a slight amount of oxidation appeared to improve the repellent quality of β-tetralol (Table 2).

TABLE 2

Repellent Action of β-Tetralol of Known Hydroperoxide Content*

Unoxidized β-tetralol (ml.)	Oxidized β-tetralol (ml.)	Hydroperoxide content (% of total)	Average period of complete protection for subjects with sweating skin (min.)
3.0	none	none	116
2.7	0.3	0.17	144
2.4	0.6	0.34	165
2.1	0.9	0.51	129
1.5	2.25	1.27	199
none	3.0	1.70	113

* Environmental temperatures: 90° F. dry bulb, 80° F. wet bulb.

As some amount of oxidation seemed desirable, attempts were made to control the process by the addition of certain antioxidants. Hydroquinone and thiourea accomplished the desired effect for a short time, but after five days the antioxidants became saturated with oxygen and the repellent activity decreased (Table 3).

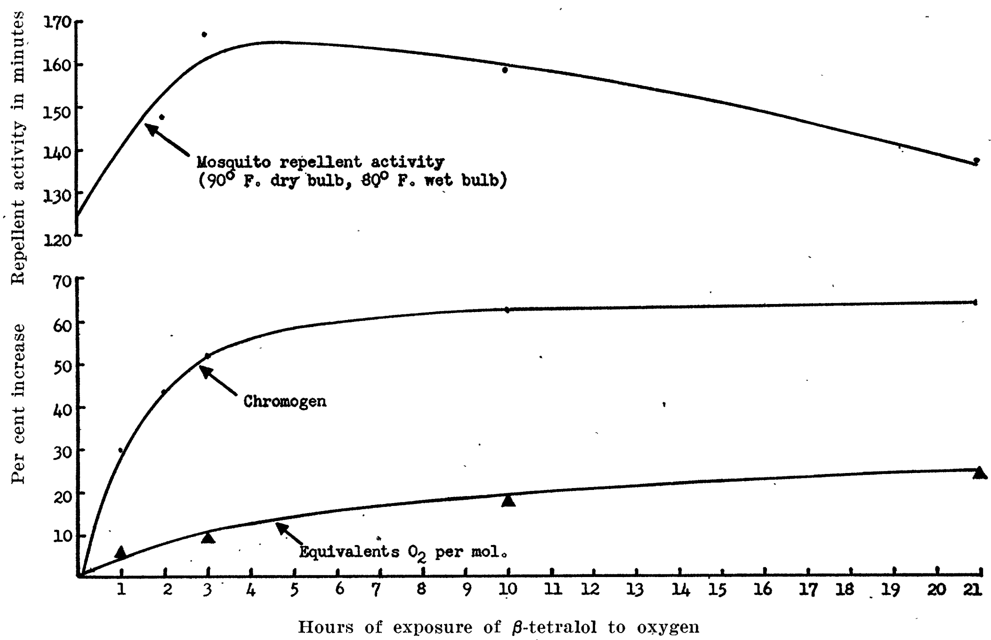

Fig. 1. Comparison between color development and oxidation of β-tetralol and its mosquito-repellent action.

In order to determine the effectiveness of NMRI-201 against a greater variety of insects, samples were sent to several agencies in the United States and in Central America. The first tests were conducted in the jungles near San Juan del Gozo, El Salvador, against the nocturnal mosquitoes, *Anopheles albimanus* and *A.*

pseudopunctipennis, and the diurnal species, *Aëdes taeniorhynchus, A. aegypti,* and *A. euplocamus.* In all jungle tests four drops of repellent were applied to the face and neck, four drops to each arm, and six drops to each leg. Four men used NMRI-201, while the fifth, serving as the control subject, applied 2-phenyl cyclohexanol. At night the control subject received the first bite 3 hours after applying the repellent. One person using NMRI-201 was bitten 11 hours after treatment, but the others were still protected when the tests were terminated at the end of 13 hours. During the day NMRI-201 repelled the *Aëdes* spp. for 3½ hours. Against these same species at Managua, Nicaragua, dimethyl phthalate protected for 47 minutes, 2-hexanediol,1,3 for 1½ hours, and oil of citronella for 18 minutes. At Jatepec Island, El Salvador, NMRI-201 protected the user from *A. taeniorhynchus* and sand flies (*Culicoides* sp.) for 5 hours and, in an uncompleted test, for 7 hours from *A. albimanus* and *A. pseudopunctipennis.*

TABLE 3

EFFECT OF ANTIOXIDATION ON THE MOSQUITO-REPELLENT ACTION OF β-TETRALOL

Oxidation of tetralol		Antioxidant added	Period of complete protection for subjects with sweating skin
Original exposure to O_2 (hrs.)	Subsequent exposure to air at 37° C. (hrs.)	(% by wt.)	(min.)
0	0	none	119
2	44	none	139
2	350	none	119
2	44	1.6 hydroquinone	177
2	350	"	121
2	44	1.6 thiourea	174
2	350	"	119
2	44	1.6 quinhydrone	131
2	350	"	113

Similar reports from the United Fruit Company at Manila, Costa Rica, confirmed the effectiveness of this new repellent against *A. albimanus.* At Almirante, Panama, *Mansonia tittilans* was repelled for more than 5 hours in uncompleted tests. Other studies in these areas revealed that NMRI-201 would prevent the attachment of mites (chiggers) for at least 8 hours and would repel sand flies for 3½ hours. Similar protection from mites was observed at Camp Lejeune, North Carolina, and in central Georgia.

While these field tests were being conducted, the Food and Drug Administration, Federal Security Agency, studied the toxicity of NMRI-201 and of β-tetralol and found that, although acute toxicity tests were generally favorable, NMRI-201 produced some toxicological manifestations upon continued administration to rabbits. Since the same results had been obtained with β-tetralol alone, it was apparent that this compound was the toxic fraction of NMRI-201. However, in view of the extended effectiveness of the repellent, it was concluded that NMRI-201 must be considered for adoption unless primary irritation was of high incidence. From the data on laboratory and field tests, primary irritation to the skin of varying degrees was noted in 6 of the 59 subjects (Table 4).

In view of these findings a substitute for β-tetralol was obviously needed. A series of other naphthol derivatives were synthesized and tested in the laboratory. While most of these compounds had repellent properties and many of them produced no skin irritation, they could not be used because they were either too costly to produce or were not feasible for large-scale production. Modifications, particularly the further hydrogenations, of 2-phenyl cyclohexanol were then studied. A mixture (NMRI-448), consisting of 70 per cent 2-phenyl cyclohexanol and 30 per cent 2-cyclohexyl cyclohexanol (by volume), was found even more effective than NMRI-201. Further field tests in the tropics have verified these laboratory findings (Table 5).

TABLE 4

INCIDENCE OF SKIN IRRITATION BY REPELLENT NMRI-201

Location	No. of subjects	Skin response: None	Tingling sensation	Warm sensation	Erythema
NMRI	10	8	1	1	0
Palmar, Costa Rica	2	1	0	0	1
Manila, Costa Rica	25	23	1	1	0
Almirante, Panama	13	13	0	0	0
Good Hope, Costa Rica	1	0	1	0	0
Central Georgia	1	1	0	0	0
San Juan del Gozo, El Salvador	4	4	0	0	0
Jaltepec Island, El Salvador	2	2	0	0	0
Managua, Nicaragua	1	1	0	0	0
Totals	59	53	3	2	1

TABLE 5

COMPARISON OF THE REPELLENT ACTIVITY OF NMRI-448 AND NMRI-201 IN TROPICAL FIELD TRIALS

Species repelled	Average period (hrs.) of complete protection afforded by NMRI-448	NMRI-201
Mosquitoes:		
Aëdes aegypti	5	3.5
A. taeniorhynchus	5–6	3.5–4
A. euplocamus		3.5
A. augustivitatus	10*	11*
Anopheles albimanus	11*	11*
A. darlingi	9*	
A. pseudopunctipennis	10*	
Psorophora ferox	10*	
Uranotaenia spp.	10*	
Bedbugs: *Cimex* sp.	12*	12*
Sand flies: *Culicoides* sp.	4*	3–7
Mites: Chiggers	8*	8*

* Indicates tests terminated at such time without any insect bites.

Although NMRI-448 has not been used by large numbers of persons, no cases of skin irritation have been observed. Toxicological tests by the Food and Drug Administration are incomplete, but their experiments indicate the likelihood that the compound will be acceptable for application to the skin.

Passage of the Ring Spot Virus Through Mazzard Cherry Seeds

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Among unbudded Mazzard cherry seedlings in the nursery row occasional seedlings showing ring spot and mottled patterns in their leaves are a common occurrence in western nurseries. When buds were taken from such mottled trees and placed in peach, typical ring spot symptoms, as described by the author (*1*), developed. Although postemergence infection of the seedlings in the nursery row could not be ruled out, the question of seed passage was raised.

In 1943 Mazzard cherry seed was purchased from a western grower who supplies seed to the nursery trade. The seed was taken from a mixed lot collected from stray and pollinator trees scattered through a commercial orchard of sweet cherries. The fruit on the Mazzard trees varied in color, size, shape, and ripening date; the size of the pits was also variable. It is not known whether these trees were planted as pollinators or were the result of sweet cherry on Mazzard rootstock, where the sweet cherry top died and the rootstock grew into a tree. Another lot of seed (supplied by E. L. Reeves) was from a Canadian source tree of Stark's Gold (a large-fruited yellow Mazzard) which had given uniform seedlings without evidence of ring spot symptoms in their leaves. A third lot of seed was collected from a Mahaleb cherry tree which had been previously experimentally infected with the ring spot virus and which had shown characteristic symptoms for two years. A fourth lot was collected from an untreated check Mahaleb cherry which, based on visual symptoms, was virus free. All seeds were stored for 100 days in moist peat at 36°–40° F.

The seeds were planted in ground beds in a lath house at the Citrus Experiment Station in April 1944, and growth proceeded rapidly. By 1 June scattered seedlings in the commercial Mazzard lot were showing leaves with crowded ring patterns. Since the seeds were planted closely, the seedlings became crowded, resulting in dwarfing of some. Counts were made, including only those 6 inches or more in height. Of the 467 seedlings counted, 25 were affected with ring spot. Some of these were strikingly affected with vivid, crowded, chlorotic ring patterns, necrotic rings, shot hole, and lace leaf. Others were less affected with chlorotic spots, rings and concentric rings, and oak-leaf patterns. In all cases the size of the rings was in inverse proportion to the number present—the greater the number, the smaller the diameter. No evidence of ring spot was seen in 90 seedlings resulting from the Stark's Gold seed, in 167 seedlings from the Mahaleb experimentally infected with ring spot, or in 120 seedlings from the visually healthy Mahaleb tree. Since these seedlings were grown in the same bed, they serve as a check to show that the Mazzards were not infected from an outside source.

In October 1944, 6 of the 25 Mazzard seedlings showing ring spots were selected, and 2 buds from each placed in each of 2 Hale peach nursery trees. A parallel series was set up with buds from 6 of the Mazzard seedlings showing no evidence of ring spot. In April 1945, 7 of the 12 peach trees inoculated, and representing 5 of the 6 symptom-bearing Mazzards, had developed typical ring spot but varied in type and severity with the different source seedlings. Most of the infected Mazzard inoculum buds died shortly after insertion, which may account for failure of infection in 5 of the Hale peach trees. It is also possible, since the Hale peach nursery stock was obtained on the open market, that the 5 trees failing to develop symptoms were already carrying the ring spot virus and would develop no symptoms following inoculations. None of the peach trees budded from normal-appearing Mazzard seedlings developed any symptoms of ring spot, although all bore living Mazzard buds.

All of the cherry seedlings were moved to the nursery row and observed for symptoms during the growing season of 1945. Twenty-four of the original 25 Mazzards showing rings survived, about one-half of them developing good ring spot symptoms. None of the remainder of the Mazzards or of the other lots developed any ring spot.

It thus appears that the ring spot virus can invade and be carried in Mazzard cherry seeds. Since the Mazzard seed lot was a sample taken directly from the trade, it should give some indication of the percentage to be expected from seed gathered at random. The amount of the seed in this study coming from trees infected with ring spot is not known nor is the percentage of ring spot which would result if the seed were taken from a single infected tree.

Seed passage helps to explain the wide occurrence of this virus in both sweet and sour cherries. Although only a relatively few of the seeds were infected, these were sufficient to provide for widely scattered distribution in nursery stock propagated on