temperatures there is a direct relation between temperature and either synthesis or hydrolysis. At both high and low temperature extremes there are, however, two short ranges of temperature, when the rates of synthesis are above those of hydrolysis. When a hardier and a frost-susceptible variety of Cinchona tree were placed for a short time at 0° C., there was a decrease in the absolute rates of both synthesis and hydrolysis in the leaves of both varieties. On return to a higher temperature with a hardy variety, this decline was found to be a reversible one, and this variety recovered. With a frost-susceptible variety the decline was found to be an irreversible one, and the plant died (*7*). On the strength of such experiments it was suggested that a low-temperature injury or even death in a plant may be a result of interference with the normal functioning of its enzymic mechanism. That a low-temperature injury may occur above 0° C. supports this contention.

The effects of decreasing water content of leaves on synthesis and hydrolysis were determined in wilting tea leaves (*2*).

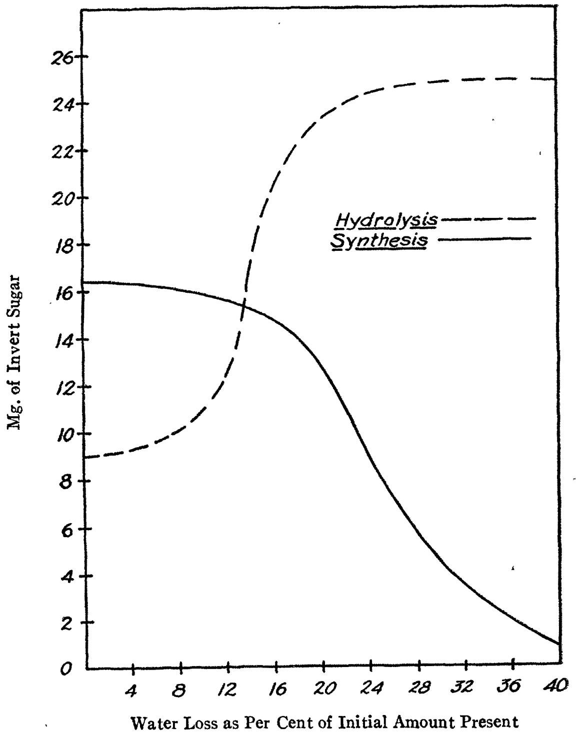

FIG. 2. Effect of water deficit on the synthesis and hydrolysis in tea leaves. (Figure from paper by A. Kursanov, *2*.)

Fig. 2 gives the data obtained. It is seen that a decrease in the water content of leaves results in a decrease in synthesis and an increase in hydrolysis. The two graphs are approximately mirror images of one another. Though the initial synthesis: hydrolysis ratio is higher in the leaves of drought-susceptible than in those of the resistant varieties of wheat, under the conditions of wilting this ratio drops off more rapidly in the former varieties than in the latter (*15*). The same was reported for the synthetic and hydrolytic action of proteases (*17*). It was suggested that the absolute value of this ratio, and the rate of its fall under the conditions of wilting, may be used in diagnosis of drought-resistant and nonresistant varieties of plants. This could be done even on plants which are in their seedling stage.

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Action of Subtilin in Reducing Infection by a Seed-borne Pathogen

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Previous studies (*2*) at this laboratory have indicated that infection by certain seed-borne pathogens may be reduced by the antibiotic action of the natural microflora of the soil. More recently attention has been concentrated on individual antagonists from the soil, especially on those showing evidence of producing antibiotic substances capable of preventing seed-borne pathogens from producing infection. A survey for such organisms in Alberta soils, conducted in collaboration with A. W. Jackson, resulted in the selection of *Bacillus subtilis* Cohn, emend. Prazmowski, as a promising antagonist. It proved to be particularly active in culture against *Xanthomonas translucens* (J. J. and R.) Dowson, a seed-borne bacterial pathogen which we had under observation. Moreover, subtilin, a known antibiotic substance (*3*) produced by *B. subtilis*, was found in plate tests to be active against *X. translucens*. Hence, this substance was chosen for the special study reported here, which has to do with the effects of subtilin on *X. translucens* and, in turn, on the disease which it produces in barley.[1]

The sample of subtilin used was kindly supplied by the Western Regional Research Laboratory of Albany, California, at the request of the National Research Council of Canada. A pathogenic strain of *X. translucens f. sp. cerealis* (*1*) was provided by W. A. F. Hagborg, of the Dominion Laboratory of Plant Pathology, Winnipeg. O.A.C. 21 barley was employed

[1] Financial assistance from the National Research Council of Canada is gratefully acknowledged.

as the host plant of the pathogen, and difficulties at first experienced in infecting it by means of seed-borne inoculum were largely overcome by the use of a modification of Wallin's (*4*) technique.

By making up the infestation medium with dilutions of subtilin in distilled water it was possible to infest seed with *X. translucens cerealis* and treat it with subtilin simultaneously. The seed was treated under partial vacuum for 1 hour and then allowed to soak in the medium for 20–24 hours. Preliminary experiments had shown that subtilin had no injurious effect on seed when applied in this way. The treated seed was then sown in 6-inch flower pots containing a 1:1 mixture of Edmonton black soil and fine sand. Twenty-five seeds were sown per pot and 200 seeds were used per treatment. One series was sown in steam-sterilized soil and another series in unsterilized soil. The pots were kept at 30° C. in constant-temperature chambers in the greenhouse for 10 days, after which notes were taken on emergence and on the number of lesioned plants. The results are presented in Table 1. The pots were then left on a greenhouse bench for 10 days longer and a second count then made. No increase in emergence occurred in the sterile series, but there was some increase in the unsterile series. The second disease count gave results comparable to the first.

TABLE 1

EFFECT OF DIFFERENT DILUTIONS OF SUBTILIN ON THE ABILITY OF *X. translucens cerealis* TO INFECT BARLEY SEEDLINGS

X. translucens cerealis in a dilution of:	Sterilized soil			Unsterilized soil		
	No. plants emerged	No. showing lesions	Diseased* (%)	No. emerged	No. lesioned	Diseased* (%)
1:1,000 subtilin	120	1	0.8	46	0	0.0
1:3,000 "	127	39	30.7	44	3	6.8
1:5,000 "	124	52	41.9	59	15	25.4
Sterile dist. H_2O	83	39	47.0	49	10	20.4

$$* \frac{\text{No. of seedlings showing lesions}}{\text{No. of seedlings emerged}} \times 100.$$

It will be noted from the above table that *X. translucens cerealis* exposed to a 1:1,000 dilution of subtilin was largely inactivated as measured by its ability to cause infection in barley seedlings. Comparable infection trends were obtained in sterilized and unsterilized soil. The consistently lower percentages of infection in the unsterilized as compared with the sterilized soil are probably attributable to the antibiotic action of the natural soil microflora, as has been reported for certain diseases of flax (*2*). The natural soil microflora is probably also responsible for the low degree of emergence in the unsterilized series, since the seed was sown in a soaked condition and kept at 30° C., which would make it quite susceptible to such action. The infection ratings in the test were based on the number of seedlings emerged. It has been shown by Wallin (*4*) and confirmed by our experiments that *X. translucens* causes a reduction in emergence of barley. Had our infection ratings taken reduced emergence into consideration, they would have been still more striking.

The results of the above experiment show that subtilin is effective in reducing infection of barley by *X. translucens cerealis* when applied as indicated at the time of infestation of the seed. It is desirable to know whether it can destroy inoculum applied to the seed before as well as at the time of treatment. Other experiments at this laboratory had shown that seed artificially infested with *X. translucens cerealis* and then dried would still produce infection when sown. Accordingly, seed was artificially infested with *X. translucens cerealis* alone and then dried for 4–5 days at room temperature. This pre-infested seed was then treated with 1:1,000 subtilin by two methods: (1) under partial vacuum for 1 hour and then allowed to soak in the solution for 20–24 hours, and (2) under partial vacuum for 15 minutes. The seed thus prepared, together with an untreated check, was then sown at the rate of 350 seeds per treatment in a 1:1 sterilized soil and sand mixture and kept at 30° C. in constant-temperature cabinets in the greenhouse. Emergence and disease counts, taken 7 days later, are presented in Table 2.

TABLE 2

EFFECT OF TREATING BARLEY SEED, ARTIFICIALLY INOCULATED WITH *X. translucens cerealis*, WITH A 1:1,000 DILUTION OF SUBTILIN

Treatment	Seedlings emerged	Seedlings lesioned	Diseased (%)	Emergence (%)
Vacuum, 1 hr.; soaked, 20–24 hrs	165	29	17.6	47.1
Vacuum, 15 min	261	90	34.5	74.6
Check	249	103	41.4	71.1

These results show that subtilin will act to reduce infection when applied to seed artificially infested with *X. translucens cerealis* before treatment. The low emergence in the first treatment is probably due to the effect of the two rather severe vacuum treatments, one during the infestation and the other in the application of subtilin.

It is not implied that the results here reported are immediately applicable in the practical treatment of seed. However, they do indicate that seed treatment with antibiotics may, with future refinements of technique, have some possibilities of a practical nature.

The methods used here would seem to provide results that are more reliable for assessing the practical value of antibiotic substances to be used against seed-borne bacterial pathogens than are those obtainable from plate tests. Limitations of materials have so far prevented the use of other methods of determining the practical value of subtilin in relation to the treatment of seed.

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