Morphological resistance of some of the Gramineae to the wheat stem sawfly (Cephus Cinctus Norton)
by George R Roemhild

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
Plant resistance to insects is not a new concept since first reports date back more than 150 years. By the time means had been developed to fully utilize this method of control spectacular results in insecticide control had become prominent and overshadowed it. We are now aware that it may be one of our cheapest and best methods of control over a period of years. The wheat stem sawfly (Cephus cinctus Norton) was originally a parasite of the native prairie grasses but migrated and adapted itself to the , wheat plant. To date, the only satisfactory means of control has been through the development of resistant varieties of wheat such as Rescue P.I. 12435. This study was undertaken to determine the exact nature of the resistance of Rescue and other cereal crops. Two oat, two barley, and four wheat varieties were studied. At regular intervals during the growth of the plants samples were taken and compared histologically for differences in structure that could be associated with resistance. It was shown that resistance in the same variety could be changed by entering the environment i.e. shading. In the case of the solid-stemmed wheats the environmental changes produced changes in the thickness of the cell walls of the parenchyma tissue. . Various varieties reacted in varying degrees to environmental change but there was a direct relationship between the thickness of their cell walls and larval mortality. Barley and oats were less affected morphologically by changing environment than wheat. These outside changes did, apparently, change the growth rate of barley plants and their desirability as ovipositing sites to the extent that differences were observed in damage. A morphological study of the oat plants indicated that resistance in this genus was probably due to something besides structure.

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INTRODUCTION

Insect resistance has been noted in certain crop plants for more than a century and a half. Probably the first report on resistance was made by Isaac Chapman in 1785 when he noted that certain varieties of wheat had possibility of resistance to the hessian fly, Phytophaga destructor Say. Fifty years later, in 1831, Lindley reported an apple variety, Winter Majetin resistant to the woolly apple aphid, Eriosoma lanigerum Hausm. Little was done in this field from this time until 1930 when the sciences of genetics and plant breeding had developed to a point where they could be used in developing resistant varieties of crops. During the decade 1930 - 1940 Snelling (1941) reported over one hundred sixty-three papers describing more than one hundred plants resistant to as many insects. These were more papers than had been published during the preceding one hundred and twenty years. With the advent of World War II and the synthesis and subsequent release of numerous potential insecticides, attention was drawn from crop resistance into the more spectacular insecticide field. At the present time there appears to be a gradual return to the realization that insecticidal control is not the answer to all our problems and that biological methods deserve a consideration in almost any entomological research program.

The precise value of biological applications must, of course, be determined by the nature of the particular problem in question.

The wheat stem sawfly, Cephus cinctus Norton, was first reported in 1872 from Colorado. In 1890 it was found in California and the following year in Nevada and Montana. Riley and Marlatt who picked it up in the last two states predicted that, "it may be expected at any time to abandon its natural food plant, the grass, in favor of the small grains". In 1895 this prophesy was fulfilled and Fletcher (1897), in his report to the Dominion noted slight damage to wheat at Indian Head, Northwest Territories. The next three years saw the insect reported from the Dakotas, Oregon, and Wyoming in the United States and from Manitoba and Saskatchewan in Canada. In 1908 the farmers of the Canadian prairie provinces were becoming alarmed at the rapid rate of increase in damage to wheat and the next year a survey of one hundred fields north of Minot, North Dakota, showed damage in all fields except one. By 1919 damage was general throughout North Dakota, parts of Montana, western Minnesota, and the southern Canadian prairie provinces. In 1952 a survey conducted by the Bureau of Entomology and Plant Quarantine reported the sawfly present in all counties of North and South Dakota, all counties in Montana east of the Continental Divide, all but nine counties in Wyoming, all but one county in Nebraska, and in twelve counties in the Red River Valley in northwestern Minnesota. In Montana and North Dakota where wheat is the principal cultivated host plant, Davis (1953) estimated over 5,100,000 acres infested and the total loss, due to this insect, over

7,895,000 bushels of grain.

For a number of years agencies in Canada and the United States have been conducting experiments aimed at sawfly control through various means. The development of resistant wheats and parasite studies have been the two primary fields of research. The wheat-breeding program has been successful in that a number of resistant varieties have been produced. One of these, Rescue, a solid-stemmed, hard red spring wheat has been grown on thousands of acres in sawfly infested areas.

The resistance exhibited by Rescue has been commonly thought to be associated with stem solidness and evidence would tend to bear this out. In cloudy wet years the plants exhibit a tendency to become hollow with a resulting increase in sawfly damage; if the weather is clear and dry, the reverse is true. These modifying effects of environment are well demonstrated in the work done by Farstad and Platt (1946) on barley and by Platt, Farstad and Callenbach (1948) on wheat. It was noted in these trials that although relative resistance of varieties remained the same in relation to one another, the whole range shifted according to the location of the test plots. Resistance was also found to vary where soil types were different. Kolar (1952) felt that sunlight intensity as well as moisture was responsible for variations in resistance of solid-stemmed wheats to the wheat stem sawfly. Germar (1934) pointed out that light controls the amount and place of silica deposition which, in turn, is very closely related to the predisposition of the plant to infection by certain fungus diseases.

McColloch (1923) stated that silica, when given to the plant in the form of the sodium salt, was found to induce resistance to the hessian fly.

Plant structure and composition have often been suggested as major influents in contributing to resistance although in many cases it has not been established if the changes in these factors are brought about, as in the case of silica, by differences in light intensity or other ecological factors. Emery (Snelling, 1941), in his work on resistance of alfalfa to pea aphid, Macrosiphum pisi Kltb., stated that the degree of resistance of a particular variety is correlated, in part, with the amount of sclerenchymous tissue and of lignin in the walls of the rays of the growing shoot. He also stated that variations in temperature and light caused fluctuations in resistance. The resistance of red clover leaves to mechanical puncuring is inversely proportional to the susceptibility of the plant to damage by the leaf hopper, Empoasca fabae Harr., according to Jewett (1935). Blanchard (1943) stated that the darker the color of soy bean leaves the more resistant they are to attack by the Japanese beetle, Popillia japonica Newm. Bramstedt (1939) showed, on a histological basis, that characteristic changes in the tissues of apple seedlings provide a definite indication of their resistance or susceptibility to the woolly apple aphid. Lees (1926) pointed out that the water content of the plant is an important factor in bringing about varietal differences in resistance to the plum curculio, Conotrachelus nenuphar Herbst., and the painted hickory borer, Megacyllene caryae Gahan. Certain specialized plant products such as oils, gums, resins, and alkaloids are claimed by Snyder (1921) to give resistance to termite attack.

Varietal and growth characteristics of the plant and their relation to insect development and activity have been found by some authors to exert an influence on resistance. Cunliffe and Fryer (1925) noted that the two and three leaf stage of oats is the most susceptible to the frit fly, Oscinella frit Linn. Painter (1930) revealed that the position of the hessian fly egg on the wheat plant was correlated with larval mortality. No larvae survived from eggs laid on the outer leaf, six per cent survived from those on the second leaf and forty-five per cent from those on the third or central leaf. He suggested that inability of the larvae to start feeding on the outer leaves may be due to the presence of cellulose or some condition associated with it. The height of the ligule also contributes to hessian fly resistance according to McColloch (1923). The higher the ligule the greater the barrier to the migration of the larvae from the top to the bottom of the plant where they can begin feeding. In other work, on corn ear worm, Heliothis armigera Hbn., he found that the date of planting had an important effect. He also noted that the particular variety of corn had an influence on the number and location of eggs laid.

It has been noted that there are many possible causes of resistance in plants to insect attack. Snelling (1941) lists fourteen different classifications of resistance. Painter (1951) gives thirtytwo different plant, insect and environmental factors which may influence resistance and further states that many of these factors are interrelated.

It has been known for a number of years that factors other than stem-solidness must effect resistance to sawfly in the Gramineae. Barley, which is hollow, shows a great deal of varietal variation in resistance. Oats, which is also hollow, exhibits an almost absolute resistance to sawfly attack. The exact nature of these resistance mechanisms are not known and it has never been determined if the resistance in oats is an intensification of the resistance mechanism found in barley or is of a different nature.

It is the purpose of this investigation to compare the structural characteristics of wheat, oats, and barley to determine if structure can be related to resistance.

For their aid, encouragement, and help I wish to thank the following personnel of the Entomology Research Section, Agriculture Research Service; Mr. P. Luginbill Jr., Mr. E.G. Davis, Mr. W. Baker, Mr. H.W. Somsen, and the following personnel of Montana State College; Mr. J.H. Pepper, Mr. J.A. Callenbach, and Mr. E.B. Hastings. Thanks are also due to my wife Mary for her aid and encouragement.

TECHNIQUE

The experimental plots used in this work were located about twelve miles east of Choteau, Montana, in a region where dry-land farming is practiced. Two randomized complete blocks were laid out containing four replications per block of the eight grain varieties grown. Each varietal plot was made up of three six-foot rows which were spaced at one-foot intervals.

Since previous experiments had shown that shaded wheat had a tendency to be damaged more by the sawfly, muslin covered frames were placed over one block to reduce resistance and obtain higher rates of sawfly cutting.

Four varieties of spring wheat were planted: Rescue P.I. 12435, a hard red solid-stemmed vulgare wheat, Thatcher P.I. 10003, a hard red hollow-stemmed vulgare wheat, Golden Ball C.I. 6227, a solid-stemmed durum wheat, and No. 17407 P.I. 113953, a semi-solid pyramidale wheat. Rescue, Golden Ball and No. 17407 had shown good sawfly resistance qualities in previous tests while Thatcher had been highly susceptible.

Two barley varieties were planted: Trebi C.I. 936, which was cut two and one-tenth per cent in tests conducted by Farstad and Platt (1946) and Hannchen C.I. 4841, which was cut twenty-one and seven-tenths per cent in the same tests.

Two varieties of oats, Vicland C.I. 3611, a small-stemmed variety, and a selection from an unknown variety of large-stemmed side oats, were used to determine if stem size would make any difference in susceptibility.

Portions of some of the oat and barley varieties were covered, in 1952, with glass cages containing sawflies to insure a high percentage of infestation. This was necessary because certain varieties appear to be non-attractive to the sawfly if a more preferable variety is available. Cages were left on for an average period of two days.

From June 20, when the eggs were being laid, until August 20, when the plants were mature, random samples of ten plants were taken at weekly intervals from each plot for microscopic study and for dissection to determine the degree of infestation and mortality at progressive stages of growth. Observations on number and location of eggs and larvae, plant size, etc., were recorded. At the end of the growing season each three-row plot was divided into nine equal sections. Five of these sections were randomly chosen and analyzed in their entirety for the number and location of eggs laid, number of plants cut, number of dead larvae, number of nodes tunneled and the height of the plants.

For microscopic sectioning two methods of dehydration and clearing were used; the regular alcohol-xylene series and a graded series of n-butyl alcohol as recommended by Sass (1940). The latter resulted in less brittleness as well as a saving in time. After embedding in paraffin and sectioning, staining was done in aqueous safranine and alcoholic fast green. Safranine stained the lignified, suberized and cutinized tissue and fast green the remainder.

An ocular micrometer was used to take measurements of the following anatomical features at weekly intervals; thickness of the nodal plate, the thickness of the lignified layer immediately below the node and the thickness of the walls of the cells making up this layer, the length and diameter of these cells, the length, diameter, and wall thickness of the parenchyma cells in the top one-third, center one-third and bottom one-third of the internode, and the thickness of the parenchyma layer in the hollow-stemmed varieties. Notes were also recorded as to whether the parenchyma was dense and organized or labile in appearance.

EXPERIMENTAL RESULTS

The various percentages of stems infested, cut, girdled but not broken over, and with live larvae at harvest are given in Table I for the eight grain varieties grown in shaded and open plots for the years 1952 and 1953. The data presented in this table were taken from the five randomly chosen sections from each varietal plot.

Infestation was higher in all cases in 1952 than in 1953. With the exception of the two oat varieties it was likewise greater in all open than shaded plots. In 1952 Vicland oats had 93.7 per cent infestation in the open as compared to 97.6 per cent in shaded plots. In 1953 the trend was reversed and Vicland was infested 29.6 per cent in the open and 11.5 per cent in the shade. Side oats had 60.2 per cent infestation in the open plots in 1952 and 99.8 per cent in shaded plots, while in 1953 the infestation was only slightly lower (1.3 per cent) in open plots than in shaded plots (1.5 per cent). The percentage of stems cut, based on the total number of stems infested, was higher in Thatcher than in any of the other varieties in both shaded and open plots for both years. Both years cutting was higher in all shaded wheat plots than in open plots. The greatest difference in cutting between shaded and open plots of the same variety occurred in Rescue where it was 62.1 per cent higher in shaded plots in 1952 and 40.2 per cent higher in 1953. The least difference was shown by Thatcher. The rest of the wheats behaved as did Rescue in that there was a greater percentage difference in cutting between shaded and open plots in 1952 than

Table I. Percentage stems infested, cut, girdled but not broken over, and with live larvae in shaded and open plots for the years 1952 and 1953.

	O-Open S-Shade	Year	Infested %	Cut* %	Girdled but not broken over* %	With live larvae* %
Thatcher (Vulgare wheat)	O	52	100.0	85.9	1.6	87.5
		53	68.0	86.4	0	84.0
	S	52	98.7	92.4	2.2	94.6
		53	50.1	85.6	0	85.6
Rescue (Vulgare wheat)	O	52	99.9	9.0	5.2	14.2
		53	44.2	27.7	1.2	28.8
	S	52	99.7	71.1	.1	71.2
		53	37.3	67.9	.7	68.6
Golden Ball (Durum wheat)	O	52	100.0	5.3	11.9	17.2
		53	66.6	39.9	6.9	46.8
	S	52	100.0	24.4	6.1	30.5
		53	46.7	48.6	9.3	57.9
No. 17407 (Pyramidale wheat)	O	52	100.0	15.5	5.4	20.9
		53	57.7	58.4	6.4	64.8
	S	52	100.0	33.0	3.9	36.9
		53	40.1	65.2	3.5	70.2
Hannchen (Barley)	O	52	90.2	42.5	2.6	45.1
		53	5.0	13.0	0	21.7
	S	52	42.3	36.7	0	36.7
		53	2.6	22.2	0	22.2
Trebi (Barley)	O	52	36.2	11.4	.1	11.5
		53	.6	0	0	0
	S	52	18.1	1.9	0	1.9
		53	.3	0	0	0
Vicland (Oats)	O	52	93.7	0	0	0
		53	29.6	0	0	0
	S	52	97.6	0	0	0
		53	11.5	0	0	0
Side Oats (Oats)	O	52	60.2	0	0	0
		53	1.3	0	0	0
	S	52	99.8	0	0	0
		53	1.5	0	0	0

*Based on total infested.

in 1953. The greatest differences in cutting between years in plots treated alike occurred in No. 17407 where 42.9 per cent more cutting occurred in open plots in 1953 than in 1952. In shaded plots 32.2 per cent more cutting occurred in 1953 than in 1952. Again Thatcher was the least affected.

In 1952 the two barley varieties gave results in exact opposition to the wheats in that there was more cutting in the open than in the shade. In 1953 no cutting occurred in the Trebi, probably due to the low infestation, but Hannchen was cut more in the shaded than in the open plots. It should be mentioned here that at the time of harvest in 1953 the Hannchen was still comparatively green and more cutting might have resulted if harvest had been delayed.

The percentage of stems girdled but not broken over was highest in the heavy-stemmed varieties, Golden Ball and No. 17407. Open grown plants of these two varieties and Rescue showed a greater tendency to stand after girdling than did shade grown plants.

Another point revealed by the data but not included in the table was the fact that there was an unusually high percentage of larval mortality due to parasitism in Hannchen in 1952. About 4 per cent of the mortality was due to parasites in open grown plants and 7.5 per cent in the shade. Only a fraction of one per cent was parasitized in any of the other grains in 1952 and in any of the grains, including Hannchen, in 1953.

The percentage of larval mortality in the node and various

Table II. Per cent larval mortality in various regions of the stem for the different varieties under the two conditions.

Variety	% Mortality in open plots				% Mortality in shaded plots			
	Internode			Node	Internode			Node
	Top 1/3	Center 1/3	Bottom 1/3		Top 1/3	Center 1/3	Bottom 1/3	
Thatcher	T*	T	T	T	T	T	T	T
Rescue	73	27			20	20	40	20
No. 17407	68	18	9	5	40	40	10	10
Golden Ball	86	11	4		43	7	7	43
Hannchen	8		4	88		7		93
Trebi			29	71			33	67
Vicland	6	18	16	60	2	2	17	81
Side Oats			16	84	2		4	94
*T - Trace								

Table III. Numbers of eggs oviposited in various regions of the internode.

Variety	Internodes from open grown plots			Internodes from shade grown plots		
	Top 1/3	Center 1/3	Bottom 1/3	Top 1/3	Center 1/3	Bottom 1/3
Thatcher	141	27	2	51	56	2
Rescue	49	34		72	33	1
No. 17407	39	14		73	32	2
Golden Ball	49	14	3	51	36	8
Hannchen	19	11	2	8	16	5
Trebi	1	4		1	1	
Vicland	58	32	3	25	29	7
Side Oats	1			33	12	2
Total	357	136	10	314	215	27

regions of the internode of the plant for the different varieties under shaded and open conditions is presented in Table II. It should be noted that 94 per cent of the mortality occurred in the top two-thirds of the internode in open grown, solid-stemmed wheat varieties. This region is also the one where most of the oviposition takes place as shown in Table III. When these solid-stemmed wheats are grown in the shade, mortality is spread more evenly throughout all regions of the plant. This is most pronounced in Rescue which is known to 'break down' in its resistance to sawfly under shaded conditions, but is apparent to a lesser degree in Golden Ball and No. 17407 which are considered stable.

In barley and oats, all of which are hollow, most of the mortality occurred at the nodal plate or immediately above it.

As noted in Figures 1a and 1b the parenchyma of the solid-stemmed wheats showed a definite trend toward thicker cell walls. Chlorination and staining of this material indicated that the thicker walls were apparently the result of deposition of lignin within them during secondary thickening. It should be pointed out that regions of the plant where the cell walls were the thickest were also the regions where most of the mortality occurred. The cell walls of the barley and oat plants never reached the thickness nor became lignified to the extent found in wheat (Figures 1b and 1c). In fact, it would seem to be characteristic of the barley and oat plant that little or no lignification takes place within the parenchyma.

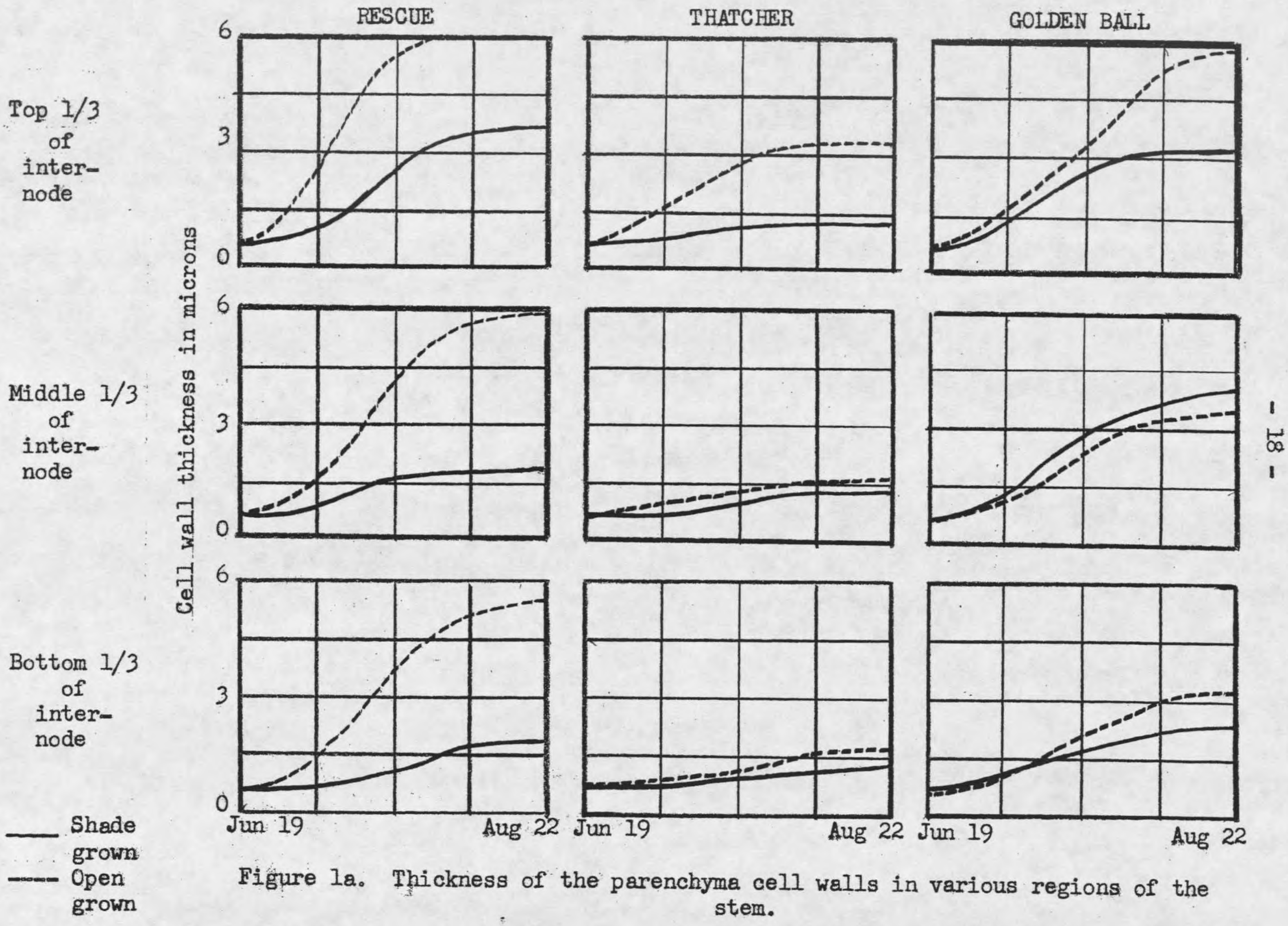

Figure 1a. Thickness of the parenchyma cell walls in various regions of the stem.

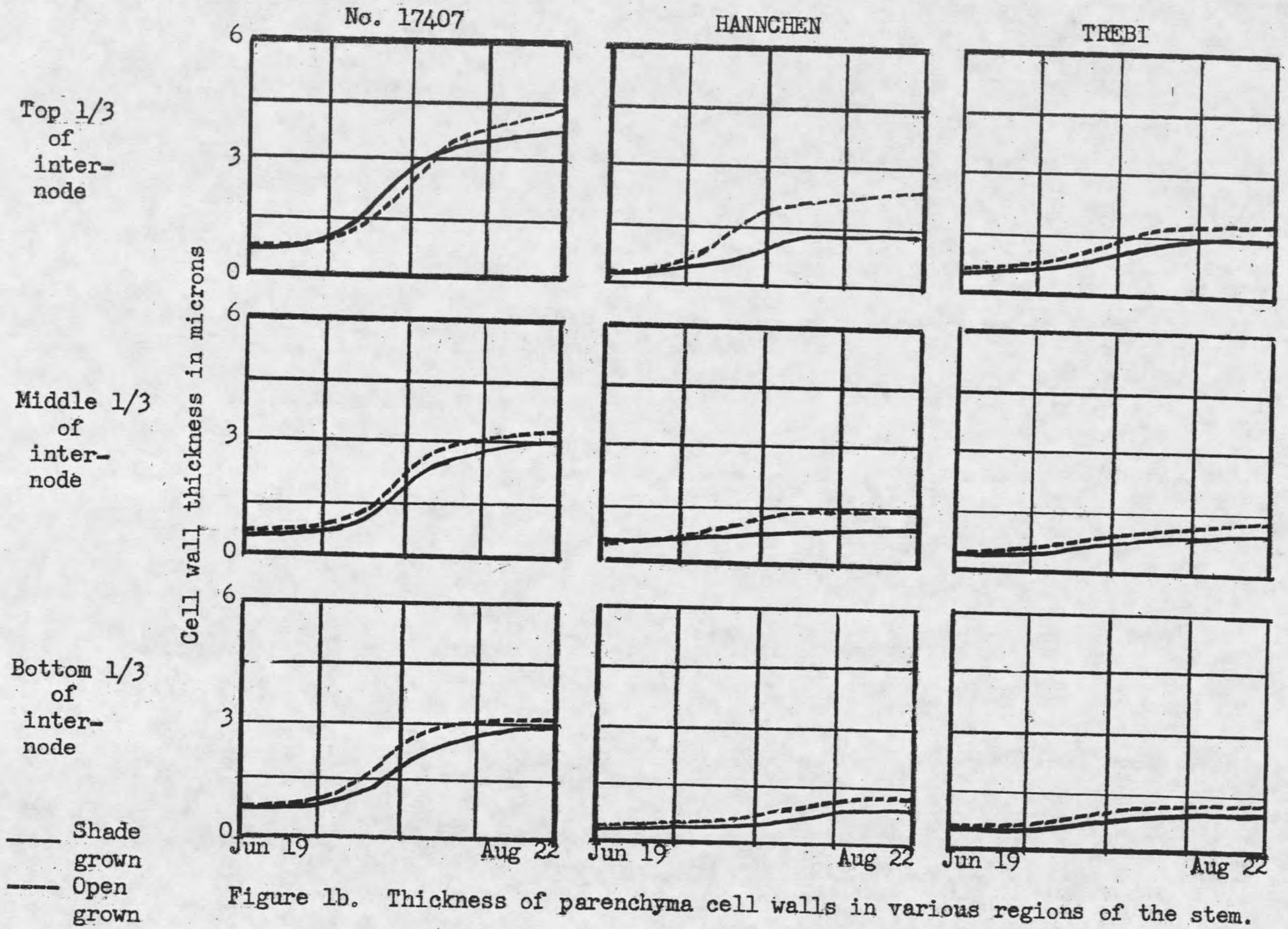

Figure 1b. Thickness of parenchyma cell walls in various regions of the stem.