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# STUDIES ON VARIETAL RESISTANCE TO STEM ROT

(Phytophthora vignae Purss) IN THE COWPEA

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#### SUMMARY.

The reaction of local and introduced cowpex varieties and some other legume species to stem rot (Phytophthora vignae) in pot tests, nursery plots and field trials is described.

On the basis of their reaction the varieties may be placed in three groups. The first includes those such as Poona and Reeves which are susceptible under all conditions of testing. The second includes Malabar, Giant and some others which are susceptible in pots but show variable reaction under other conditions. The varieties in the third class, which exhibit complete resistance, include Blackeye 5 and Havana.

It is shown that the resistance to stem rot possessed by Blackeye 5 is dominant over the susceptibility possessed by Poona.

Evidence is produced to show that control of the disease can be achieved by the use of resistant varieties either introduced from overseas or synthesised by plant breeding.

#### I. INTRODUCTION.

In an earlier paper (Purss 1957) a new disease of cowpeas (Vigna sinensis (L.) Endl. ex Hassk.) known as stem rot and the causal organism (Phytophthora vignae) were described. This paper is devoted to studies of the reaction of various cowpea varieties and other leguminous plants to the disease.

Control of soil-borne diseases such as stem rot is best achieved by the use of resistant varieties. In the case of cowpeas, which are used primarily as a green manure or fodder crop, the use of more expensive control measures could not be contemplated.

The two most popular varieties, Poona and Reeves, are both susceptible to the disease. Other cowpea varieties developed locally or introduced from other countries by the Plant Introduction Section of C.S.I.R.O. have therefore been collected and subjected to disease testing. In addition, other species of legumes considered to be of some potential importance under tropical and subtropical conditions have been screened.

This work has been carried out under three different sets of conditions—pot testing, nursery plots and field trials.

# II. POT TESTING.

The technique employed here was identical with that used in the pathogenicity tests described in the earlier paper, where inoculation was done below ground level. Briefly, seedlings were raised in 7 in. earthenware pots and when

Table 1.

Reaction of Cowpea Varieties to Stem Rot in Pot Tests and Nursery Plots.

			Pot	Tests.	Nursery Plots.	
Variety.	C.P.I. No.	Source.	No. of Plants Inoculated.	Percentage Infection.	No. of Plants Germinating.	Percentage Infection.
Arlington	9,256	Oklahoma .	. 19	100	46	94
Bayo	12,149	Cuba	. 8	100	24	91
Black		Local		100	20	100
Black	9,254	Oklahoma .	. 6	83	19	100
Blackeye 5	12,377	California .	4.0	0	79	0
Blackeye 7	12,378	California .	. 6	100	9	88
Brabham		Local	. 36	100	18	63
Brabham	9,249	Oklahoma .	. 6	100	13	100
Buff	9,247	Oklahoma .	0.0	61	23	33
Carito	12,150	Cuba		100	3	100
Chinese Red	9,259	Oklahoma .	40	0	45	2
Cowpea No. 3	10,753	U.S.S.R.	1.0	90	22	66
Cristaudo	10,.00	Local		63	109	46
C 419	12,111	India	00	100	86	26
C 442	11,043	India	-	100	16	73
C 521*	11,044	India	7.0	66	9	22
Early Cowpea	11,384	Gold Coast .		60	10	40
Early Giant	11,001	Local	0.0	100	83	76
Early Red	9,245	Oklahoma .		100	10	40
Giant		Local	0.0	95 '	94	29
Groit		Local	1.5	100	80	81
Groit	9,260	U.S.A	·	75	5	60
Iron	5,984	Texas	1 77	55	15	0
Iron	9,248	Oklahoma .		64	71	38
Local Tanganyika	8,521	Tanganyika .	1 70	50	34	48
Local Zanzibar	8,520	Tanganyika .		63	15	66
Nigro*	12,148	Cuba		0	19	0
Polon Prolific V4	9,322	Ceylon		100	8	38
Poona		Local	0.0	100	200	100
Reeves	• • •	Local	0.0	100	266	90
Rojo*	12,153	Cuba	00	0	54	2
Soutter	12,100	Local	0.0	97	139	50
Tabora 2	8,522	Tanganyika .	10	70	23	34
Victor		Local		92	81	98
X7' - 4	9,246	Oklahoma .		100	38	100
$egin{array}{ccccc} { m V}_{10} { m tor} & \dots & $	9,323	Ceylon		100	20	95
White Crowder.	9,323 9,252	Oklahoma .	.	40	11	27
0.40001	9,232 9,432	South Africa .	115	93	150	65
9500	8,086		0.1	100	39	70
37C6	0,000	South Africa .	.   41	100	00	

<sup>\*</sup> C.P.I. numbers 11044, 12148, and 12153 have been renamed Malabar, Santiago and Havana respectively by Australian authorities.

approximately four weeks old were inoculated with P. vignae growing on potato dextrose agar. Small cubes of the inoculum ( $\frac{1}{4}$  in. square) were placed against the uninjured stems just below ground level and covered with soil. The pots were kept well watered. This technique proved highly effective but to obtain consistent results testing had to be confined to the warmer months of the year.

The number of plants of each variety used was not constant because of the variable germination of the material. The shortage of seed prevented any replication in most of the varieties, but the susceptible Poona was used at check points throughout to give an index of disease distribution. This variety was completely susceptible in these tests. Where possible, the work was conducted in a glasshouse. At other times humidity was maintained by the use of frames erected over the pots and covered with transparent plastic sheeting.

Over 200 varieties and strains have already been screened in this way and a further 300 varieties are in the process of being examined. It would be of little value to list the reaction of all varieties, as the majority were unnamed lines from overseas. The reaction of most of the named varieties appears in Table 1.

The reaction of various other legumes to the causal organism of the disease has also been determined under pot conditions. Most of these legumes have a potential value in a tropical or subtropical environment. The velvet bean,

Table 2.

Reaction of Various Legume Species in Pot Tests.

Legume Species.	Variety.	No. of Plants Inoculated.	Percentage Infection.	
Cajanus cajan (L.) Millsp. (Pigeon pea)		10		
Calopogonium mucunoides Desv. (Calopo)			. 11	0
Glycine javanica L			10	0
Glycine max (L.) Merr. (Soybean)		Gaton	11	0
		Avoyelles	6	0
		Otootan	7	0
Medicago sativa L. (Lucerne)			- 11	0
Mucuna spp. (Velvet bean)		Sommerset	8	0
		Black	7	. 0
		Mauritius		• •
Phaseolus lathyroides L. (Phasey bean)			11	0
Phaseolus mungo L. (Mung bean)			10	0
Phaseolus vulgaris L. (Bean)		Black Beauty	10	0
		Brown Beauty	10	0
Pisum arvense L. (Field pea)			11	0
Pueraria phaseoloides (Roxb.) Benth. (Puer	o)		7	0
Vicia sativa L. (Common vetch)		• •	13	0
Vigna sinensis (L.) Endl. ex Hassk. (Cowpe	эа)	Poona	10	100
		Snake bean	18	100

for instance, is used as a green manure crop in place of cowpeas in some Queensland canefields. The result of inoculating these legumes appears in Table 2.

The results of these tests indicated that very good resistance to the disease was present in at least four varieties of cowpea—Blackeye 5, Havana, Santiago and Chinese Red. Of the species of legumes tested, only *Vigna sinensis* was susceptible to the disease.

#### III. NURSERY PLOTS.

In early field trials there were indications that differences between pot reaction and field reaction to stem rot could be expected. In an effort to simulate severe field conditions, nursery plots were instituted. For these plots, rows were opened up 1 foot apart and in these were buried the remains of infected plants collected either from pot tests or from diseased fields of cowpeas. The seed of the varieties being tested was then placed in these rows. This close planting gave ideal conditions for the development and spread of the disease. The plots were kept constantly moist.

Seed of most varieties was in short supply and replication had to be dispensed with. However, the susceptible Poona was sown in rows in such a pattern that every variety being tested was adjacent to it. This variety died out in all plots as a result of stem rot infection, indicating an even distribution of the disease in the nursery.



Fig. 1.

Nursery Plots. Two centre rows of Blackeye 5 bordered by rows of Poona,

Results of the nursery plot testing appear in Table 1. The indications from these tests were that certain varieties such as Malabar and Giant, while susceptible in pot tests, possessed considerable resistance in nursery plots. Other varieties, such as Blackeye 5 and Santiago, which were resistant in pots were equally resistant in the nursery. In Fig. 1 two rows of Blackeye 5 can be seen growing healthily, while on each side the rows of Poona are badly affected by the disease.

#### IV. FIELD TRIALS.

These were conducted every season from 1952-53 until 1956-57. The object was to test varieties under conditions prevailing in the field rather than in the artificial conditions of the glasshouse or disease nursery. The trials were planted in almost all cases on "infested" soil—that is, on soil that had carried a crop infected with stem rot during the previous season. The varieties used during the course of these field trials have changed to some extent as susceptible lines were discarded and seed of resistant lines became available.

## (1) 1952-53 Season.

During the 1952-53 season two trials were conducted in the Fassifern Valley. The first one was laid down at Fraserview as a grazing trial, but with the outbreak of stem rot it was possible to rate the susceptibility of the varieties to this disease. No cowpeas had been grown on this paddock for at least 10 years, although the crop was commonly used on the property. It can be assumed that the inoculum level in this area was low. The trial was replicated in four blocks.

The second trial was a variety trial planted at Teviotville on soil which was said to have carried an infected crop during the previous season. Restricted seed supplies of most varieties permitted only two blocks, with single-row plots 1 chain long, to be sown.

Table 3.

RESULTS OF STEM ROT RESISTANCE TRIALS, FRASERVIEW AND TEVIOTVILLE, 1952–53 AND 1953–54. (EXPRESSED AS PERCENTAGE OF THE TOTAL NUMBER OF PLANTS THAT BECAME AFFECTED.

Varie	ety.		Fraserview 1952–53.	Fraserview 1953–54.	Teviotville 1952–53.	Mean.
Poona			60	90	95	81.7
Reeves			37.5	83.75	92	$71 \cdot 1$
Cristaudo			0	36.25	19	18.4
C.P.I. 9432		[	6	53.75	67	$42 \cdot 3$
$\operatorname{Giant}$				11.9	10	10.9
Groit					90	90
Soutter					80	80
$\mathbf{Brabham}$					87	87
Black				98.75		98.75

Counts were made on random quadrats in each case and the results appear in Table 3.

The results of these two trials, coupled with glasshouse figures, indicated that Giant and Cristaudo, two varieties available commercially, possessed good field resistance while the remainder of the varieties were of little value. The stand produced by Cristaudo in the first trial is shown in Fig. 2.



Fig. 2.

The Cristaudo Variety of Cowpea Growing on Infested Soil.

# (2) 1953-54 Season.

In the 1953-54 season a further trial was conducted at Fraserview on the site used for the grazing trial during 1952-53. The plots used were  $1\frac{1}{2}$  chains long and 14 yards wide and the seed was broadcast by hand. Disease incidence was assessed by using random quadrat counts. The results appear in Table 3.

It is interesting to record that in the variety Cristaudo, of the 36·25 per cent. of plants finally infected with stem rot, 35 per cent. died in the seedling stage. This was not the case with the other varieties, where infection was spread over the life of the plants. It will be noted that while Giant maintained its resistance as the infection potential increased, Cristaudo did not.

#### (3) 1954-55 Season.

In the 1954-55 season a trial was conducted in the experimental area at Brisbane. This was designed firstly to compare a number of "strains" of the varieties Cristaudo and Giant obtained from various sources, and secondly, to try some of the more promising introductions, already glasshouse-tested, of which seed had become available. The varieties and strains were planted in four replicated blocks with plots of single rows 30 ft. long spaced 1 ft. apart. This trial was planted in soil that had carried an infected crop of Reeves during the previous season, but to ensure adequate infection diseased plants were spread evenly in the rows prior to sowing. Figures for the varieties appear in Table 4 and for the Giant and Cristaudo strains in Table 5.

Table 4.

STEM ROT VARIETAL RESISTANCE TRIAL,
BRISBANE, 1954-55.

Variety.				
			1.8	
			16.5	
			30.4	
			30.8	
			40.3	
			66.8	
			$67 \cdot 6$	
			70.5	
			$\bf 79 \cdot 4$	
omm	ercial)		91.5	
			96.5	
			99.9	
			100	
			100	
			commercial)	

Necessary differences for significance,  $5\%-13.0\,;~1\%-17.4.$ 

Table 5.

Reaction of Various Strains of Cristaudo and Giant to Stem Rot, Brisbane, 1954-55.

Strain.		Percentage Infection.
Giant (Commercial)	30.8	
Giant (1)		$22 \cdot 3$
Giant (2)		28.1
Giant (3)		$32 \cdot 9$
Cristaudo (Commercial)		91.5
Cristaudo (1)		$51 \cdot 1$
Cristaudo (2)		<b>56.9</b>
Cristaudo (3)		83.7

Necessary differences for significance, 5% - 13.0; 1% - 17.4.

Blackeye 5 and Malabar showed very good resistance, while Giant and Early Giant were moderately resistant. Little variation was encountered between strains of Giant, but as expected there was evidence of differing resistance between strains of Cristaudo.

# (4) 1955-56 Season.

In the 1955-56 season, trials were conducted over a range of conditions in south-eastern Queensland. As a result of previous work the following 10 varieties were selected for testing:—Poona, Reeves, Soutter, Giant, Cristaudo (1), Cristaudo (2), Early Giant, Havana, Blackeye 5, and Malabar.

Trials 1, 3 and 4 were planted in six replicated blocks, trial 2 in five. Plots were of single rows 30 ft. long. The trials were completely surrounded by rows of the susceptible check, Poona. Seasonal conditions in all cases were abnormally wet. A brief outline of each of the trials follows and the results appear in Tables 6 and 7.



Fig. 3.

Stem Rot Resistance Field Trial, Kumbia, 1955-56. In the foreground is the variety
Cristaudo and in the rear Malabar.

Trial 1 was located at Kumbia, in the South Burnett, on land which had carried two successive crops of cowpeas affected with stem rot. (In 1954-55 the variety Poona was completely destroyed.) From the time of planting in December until the end of April, over 33 inches of rain fell. Besides losses from Phytophthora vignae there were losses from stem rot causes by Rhizoctonia sp. and Sclerotium rolfsii; Fusarium wilt (F. oxysporium f. tracheiphilum) was also present. The incidence of these three diseases was slight and their damage was completely overshadowed by losses due to P. vignae. This trial represented the conditions that would occur in the field on land having a history of heavy stem rot infection.

The resistance of Blackeye 5, Havana, Malabar (Fig. 3) and Giant was demonstrated, while Cristaudo proved very susceptible (Fig. 3) and Poona was a complete loss (Fig. 4).



Fig. 4.

Stem Rot Resistance Field Trial, Kumbia, 1955-56. Varieties, from left to right, are Giant, Poona, Blackeye 5 and Havana.

Trial 2 was conducted at Moffatdale, also in the South Burnett district. Some details of this trial have already been outlined in an earlier paper (Purss 1957) because of the results obtained over the range of moisture conditions prevailing between blocks. It was located on soil into which a crop of Poona lightly affected with stem rot was ploughed just prior to planting. As the area was subject to flooding it was impossible to count emergence or assess disease incidence at regular intervals. Consequently, the losses recorded include minor losses such as plants washed out by flowing water. The ability of Blackeye 5 and Havana to withstand severe conditions induced by the flooding is demonstrated.

Table 6.

INCIDENCE OF STEM ROT AT THREE LOCALITIES IN SOUTH-EASTERN QUEENSLAND, 1955-56. (EXPRESSED AS PERCENTAGE OF PLANTS INFECTED).

Variety.		Trial 1, Kumbia.	*Trial 2, Moffatdale.	Trial 3, Redlands.	Mean.
Blackeye 5		0.9	20.0	1.5	7.5
Havana		$2 \cdot 3$	19:0	0.5	$7 \cdot 3$
Malabar		13.4	67.9	1.5	27.6
Giant		$8 \cdot 3$	62.2	5.0	$25 \cdot 2$
Early Giant		34.6	64.6	6.0	$35 \cdot 1$
Soutter		38.9	66.6	26.0	43.8
Cristaudo (1)		81.9	62.5	7.0	50.5
Cristaudo (2)		75.8	67.9	7.0	$50 \cdot 2$
Reeves		84.6	91.3	83.0	86.3
Poona		99.5	96.9	91.0	95.8

<sup>\*</sup> Based on losses from all causes (see text).

Trial 3 was located in the Redlands district, near Brisbane, on soil which had carried two successive crops of cowpea in which stem rot had occurred only in patches. Dry conditions were experienced during the first month but thereafter the trial was subjected to rainfall conditions similar to those encountered in Trials 1 and 2. The results are typical of those normally associated with a low inoculum level in the soil and show the resistance of Cristaudo under such conditions.

Trial 4 was conducted at Fraserview in the Fassifern Valley on the site used for the trials in the 1952-53 and 1953-54 seasons. The area was planted to a susceptible variety in the 1954-55 season and complete loss from the disease was reported. Very wet conditions were experienced throughout the trial, making emergence counts impossible, and results are therefore expressed as the total final stand (Table 7). Striking differences in susceptibility between

Table 7.

STEM ROT RESISTANCE TRIAL No. 4,
FRASERVIEW, 1955-56.

Varie	Total Final Stand	
Havana	 	371
Blackeye 5	 	337
Malabar	 	46
Soutter	 	49
Giant	 	23
Cristaudo (1)	 	8
Cristaudo (2)	 	4
Early Giant	 	2
Reeves	 	5
Poona	 	0

varieties under these conditions of heavy inoculum level and heavy rainfall bordering on flood conditions are revealed. The resistance of Blackeye 5 and Havana is demonstrated.

### (5) 1956-57 Season.

In the 1956-57 season a further trial using four replications was conducted at Kumbia on the land previously used for such trials. Conditions during this season were exceptionally dry. The results given in Table 8 reveal the field resistance of Santiago.

Table 8.

STEM ROT RESISTANCE TRIAL,
KUMBIA, 1956-57.

Variety.	Percentage Infection.	
Poona		97
Blackeye 5		0
Havana		0
Malabar		3
Santiago		$0 \cdot 2$
C.P.I. 9322		58
Giant		$3 \cdot 4$
	!	

#### V. BREEDING FOR DISEASE RESISTANCE.

Early in the testing programme it became apparent that the popular Poona variety was extremely susceptible and that new varieties would need to be introduced or synthesised by plant breeders. In an attempt to obtain simple information on the inheritance of disease resistance, Poona was crossed with the resistant Blackeye 5. The F<sub>1</sub> generation was tested immediately and backcrossed to Poona. This backcrossing programme has continued, the ultimate objective being a resistant backcross Poona. All testing done has been in pots, the plants being individually inoculated with *P. vignae*. The results of these inoculations are summarised in Table 9.

Table 9.

RESULTS OF INOCULATING VARIOUS GENERATIONS OF A POONA AND BLACKEYE 5 CROSS WITH P. vignae.

Generation.	No. of Plants.	No. Healthy.	No. Diseased.
Blackeye 5—Parent	10	10	0
Poona—Parent	10	0	10
Poona x Blackeye 5—F <sub>1</sub>	10	10	0
$-F_2$	109	83	26
1st B.C.	83	48	35
2nd B.C.	70	41	29

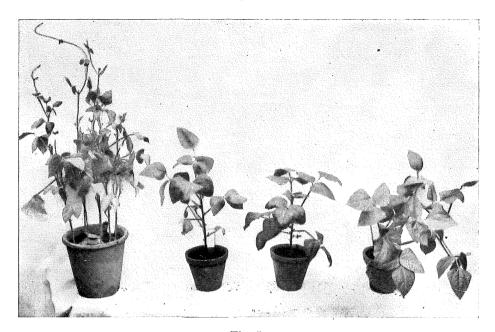
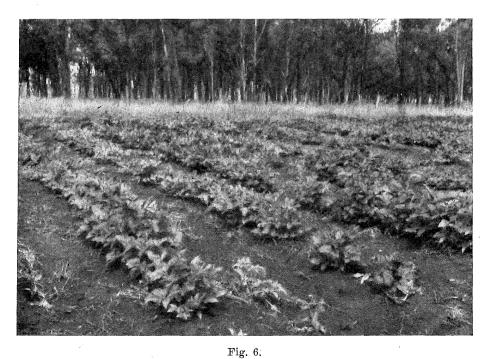


Fig. 5. The Parents and Progeny of the Poona x Blackeye 5 Cross. From left to right—Poona, two  $\mathbf{F}_1$  plants, and Blackeye 5.



General View of First Backcross Selfed Lines of the Poona x Blackeye 5 Cross Growing on Infested Soil in the Field.

The two parents and two of the  $F_1$  plants are shown in Fig. 5, and Fig. 6 is a general view of some of the 1st backcross selfed lines growing on infested soil.

The results indicate that the resistance to stem rot possessed by Blackeye 5 is dominant over susceptibility.

#### VI. DISCUSSION.

The most striking feature of these results is the difference in reaction in some of the varieties under glasshouse, plot and field conditions. The varieties can be roughly grouped into three classes.

In the first are included those that are very susceptible under all conditions of testing. Into this group such varieties as Poona, Reeves, Black and Arlington can be placed.

In the second class are varieties which are very susceptible in pots but which show variable reaction under other conditions of testing. Into this group are placed such varieties as Malabar and Giant, which possess very good "field resistance", and Soutter and Early Giant, which show this to a lesser degree. The variety Cristaudo also comes into this group; its behaviour will be referred to later. It is, within this class that we find the greatest effect of environmental conditions, such as previous history of the land in respect of number of infected cowpea crops carried, and seasonal conditions such as high rainfall and flooding.

The third class contains those varieties which maintain resistance under all manner of conditions. Included here are Blackeye 5, Havana and Santiago. Chinese Red, which withstood the glasshouse testing, probably could be included.

That such a grouping is possible would indicate that there are at least two distinct types of resistance. On the one hand there is the type possessed by the group containing Blackeye 5, which appears to be almost an immunity. On the other hand is the resistance possessed by such varieties as Malabar and Giant, a resistance best described as a field resistance which breaks down when conditions are very favourable to the pathogen and when the inoculum potential is high.

Reference has already been made to the build-up of the disease which occurs when susceptible varieties are grown season after season on the same ground (Purss 1957). The results of the trials during 1956-57 are very striking in this regard. Trial 1 at Kumbia (Table 6) was carried out on soil which had carried a very badly affected crop during the previous season. The trial at Redlands had carried infected crops but this infection had been somewhat restricted. The varieties Poona and Reeves were equally susceptible under both conditions of testing, while Havana and Blackeye 5 were equally resistant. Differences, however, appeared in the second group of varieties—Malabar, Giant, Cristaudo and to some extent Soutter. This was even more pronounced

in the trial at Moffatdale, where Malabar became badly affected owing to the flooded conditions.

The behaviour of the variety Cristaudo deserves some special comment because of the resistance this variety possessed in earlier trials (Purss 1953). The explanation of the failing resistance appears to lie in the build-up of inoculum levels. The difference in results at Redlands and the South Burnett (Table 6) illustrates this. The variety still maintains good field resistance where the inoculum level is low, a condition which was probably universal in 1953, but this resistance breaks down when the inoculum level is increased, as in Trial 1, 1956-57. The resistance also breaks down when seasonal conditions are extremely favourable to the disease, as at Moffatdale in 1956-57. There is also evidence of extreme variability between strains of Cristaudo in regards to their reaction to this disease. Both Cristaudo and Giant were developed by farmers for use in northern canefields and have not been intensively selected. The variety Cristaudo is not regarded favourably by agronomists and for this reason and the low order of resistance available it was not considered worthwhile to build up seed stocks of the more resistant lines.

#### VII. CONCLUSION.

The information presented indicates that the threatened elimination of the cowpea as an agricultural crop in Queensland on account of stem rot disease may be avoided by the use of resistant varieties. Of those already tested, Havana and Blackeye 5 withstand the disease under all conditions while Malabar and Giant possess resistance ample enough to withstand most conditions likely to be encountered in the field. These varieties may not have all the characteristics which made the displaced Poona variety so popular, but at least they closely approach the desired type.

It is apparent that should none of the resistant varieties already obtained be wholly suitable, the production of a suitable disease-resistant variety by plant breeders should present no insuperable problems.

Fortunately, all other tropical and subtropical legumes so far tested appear to be resistant to the disease.

#### VIII. ACKNOWLEDGEMENTS.

The assistance given by the C.S.I.R.O. Plant Introduction Section in providing seed of cowpea introductions is gratefully acknowledged. Many officers of the Agriculture and Horticulture Branches of the Department of Agriculture and Stock assisted in the conduct of the field trials. This assistance was invaluable.

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