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BERKELEY, CALIFORNIA 94720

12 December 1986

To: W. Barnett, L. Hendricks, W. Reil, R. Beede, and S. Sibbett

From: M.A. Hoy

M.A. Hoy

Re: Walnut Report

Enclosed is our report for the Walnut Board. I thought you might be particularly interested because you helped us with our project. We are excited about our results to date, and expect to submit a proposal for the next year's funding. We are taking it one year at a time!

Please give me a call (415) 642-3989 if you have questions. Again, thanks for all your help and support. We really appreciate it.

Sent to Don Pierce and John Fawcett.

*Field rate for 100 gpa is \approx 1800 ppm.
Can't relate field to lab rate yet. Hope to try actual sprayed
leaves with mummies in 1987.*

RESULTS

The Valley-Christensen colony, which was collected from two walnut orchards near Visalia in June 1985 and one orchard near Hanford in September 1985, was selected a total of 11 times (Table 1). This colony has increased its tolerance to Guthion. Thus, selection began with 4.2 ppm AI Guthion and was increased to 4.8 ppm for the third selection, then to 6 ppm for the fourth selection, to 12 ppm for the fifth through seventh selections, and to 15 ppm for the eighth through eleventh selections. At this point the selected strain's survival rate is ca. 60% while the survival rate of the base colony is ca. 21% (Table 1, Figure 1). The concentration/mortality lines for the base and selected (11th selection) colonies were conducted and are reported in Table 5. The LC₅₀ for the base colony is 10.5 ppm AI, while that of the selected strain is 17.4 ppm, which is a 1.7-fold increase in LC₅₀ (Figure 1).

Table 1. Selection of the Valley-Christensen colony of T. pallidus using the 50 WP formulation of Guthion

Selection no.	Dates 1986	Number selected	Dose (ppm)	% survival			
				selected		water controls	
				selected colony	base colony*	selected colony*	base colony*
1	21-24 Jan.	2756	4.2	-	71.8	-	98.5
2	8,10 Feb.	2150	4.2	92.8	90.2	98.0	100.0
3	15-19 Mar.	1240	4.8	80.2	-	97.0	-
4	1-2 Apr.	4000	6.0	86.2	69.0	99.0	96.7
5	17-19 Apr.	4975	12.0	47.7	29.3	99.3	98.7
6	5-7 May	1650	12.0	49.9	-	99.3	-
7	8-11 June	705	12.0	75.5	50.0	98.3	100.0
8	25-28 June	2000	15.0	43.4	8.0	97.3	98.7
9	1-5 Aug.	750	15.0	45.1	-	98.3	-
10	20,22 Aug.	1400	15.0	62.8	21.0	97.5	97.5
11	25,27 Sept.	1200	15.0	25.4	10.0	100.0	100.0
	2,5 Nov.	180	15.0	61.1	21.2	96.1	96.2

(Data from concentration/mortality test)

* Ca. 50 to 100 were tested as controls after the first selection.

During July 1986, Wilbur Reil collected Trioxys from a mixed walnut/almond orchard in Yolo County; this orchard had been treated repeatedly with Guthion for navel orangeworm in the almonds, and a colony of T. pallidus from this orchard was thus considered of particular interest. The results of selections on this colony are given in Table 2. This colony is being selected with 9 ppm, and yields survival rates of 12-22%, which is substantially lower than that of the Valley-Christensen colony (Table 1). The Yolo colony is also substantially more susceptible to Guthion than the Los Banos or Aggregate colonies. A concentration/mortality test was conducted after 3 selections, and is reported in Table 5. The LC₅₀ values of the base and selected colonies are 3.5 and 4.5 ppm, respectively. These lines were analyzed by the POLO program. Slopes are the same, but the lines are different. Thus, some selection response has occurred with the Yolo

Table 2. Selection of the Yolo County colony of T. pallidus using the 50 WP formulation of Guthion

Selection no.	Dates 1986	Number selected	Dose (ppm)	% survival			
				selected		water controls	
				selected colony	base colony*	selected colony*	base colony*
1	20,22 Aug.	937	9.0	-	21.9	-	97.5
2	25,27 Sept.	1200	9.0	11.8	-	100.0	-
3	2,4 Nov.	2000	9.0	18.0	9.7	98.7	100.0

* Ca. 50 to 100 were tested as controls after the first selection.

colony, although the amount of shift in the line of the Yolo-3 colony is still relatively small and line remains to the left of the Valley-Christensen base colony (Figure 1).

A colony was collected from the Los Banos area in July 1986 and has been selected four times (Table 3). Survival of the original colony after treatment with 9 ppm was 92.2%, which is higher than the Yolo County colony after three selections. Survival after treatment with 15 ppm was 27%, 38%, and 32% for selections two, three, and four. This survival rate is nearly as high as that of the Valley-Christensen colony after 11 selections. The Los Banos colony therefore appears particularly promising.

Table 3. Selection of the Los Banos colony of T. pallidus using the 50 WP formulation of Guthion

Selection no.	Dates 1986	Number selected	Dose (ppm)	% survival			
				selected		water controls	
				selected colony	base colony*	selected colony*	base colony*
1	20,22 Aug.	850	9.0	-	92.2	-	100.0
2	8,10 Sept.	2350	15.0	27.5	42.7	100.0	98.0
3	25,27 Sept.	1050	15.0	38.0	37.0	100.0	100.0
4	22,24 Nov.	1600	15.0	32.4	7.7	99.0	99.0

* Ca. 50 to 100 were tested as controls for selections 2 and 3; 350 were tested as controls for selection 4.

Finally, we collected T. pallidus from six orchards near Visalia, Hanford, and Gustine in September 1986. These colonies were pooled and are called the Aggregate Colony (Table 4). The first selection with 15 ppm yielded 27% survival, an impressive rate for a colony fresh from the field. In the second selection, survival with 15 ppm was 16%, which was higher than 7% for the base colony (Table 4). This colony, like the Los Banos Colony, also appears very promising.

Table 4. Selection of the Aggregate colony of T. pallidus using the 50 WP formulation of Guthion

Selection no.	Dates 1986	Number selected	Dose (ppm)	% survival			
				selected		water controls	
				selected colony	base colony*	selected colony*	base colony*
1	2,4 Nov.	2000	15.0	-	27.8	-	99.0
2	22,24 Nov.	2250	15.0	16.3	6.9	96.0	96.0

* Ca. 350 were tested as controls in the 2nd selection.

CONCLUSIONS

We are pleased with our rearing system for the walnuts, aphids, and parasites. We are able to rear four selected and four base colonies of parasites, although this is quite labor intensive. We have selected our four colonies for differing periods of time. The colony from Yolo county is clearly the most susceptible colony we have found. (In fact, there is a 5-fold difference in tolerance (LC₅₀) of the Yolo base and the Valley-Christensen colonies.) This is unexpected since it was treated with Guthion regularly. In contrast, the Los Banos colony and the Aggregate colonies are much more tolerant of Guthion even without selection, and we hope that their resistance levels will increase with selection during the next six to nine months.

The Valley-Christensen colony, which has been selected a total of 11 times, has responded with an increase in LC₅₀ value of ca. 1.7-fold. The base colony's LC₅₀ value was 10.5 ppm, while the selected colony's LC₅₀ value is 17.4. It is unclear whether this increase in concentration/mortality line is sufficient to allow the colony to survive Guthion applications in the field. However, we expect to continue selection with this colony, and with the Los Banos and Aggregate colonies, which tolerated Guthion very well in their preliminary tests, until we are sure that no further increase in tolerance occurs. The next three months should determine how high the resistance will go in the Valley-Christensen colony.

Table 5. Concentration/mortality responses of the Valley-Christensen and Yolo colonies of T. pallidus

Colony	No. tested	LC ₅₀ (ppm)	95% C.L.	LC ₉₀ (ppm)	95% C.L.	Slope (<u>+</u> S.D.)
Valley-Christensen- Selected-11	1240	17.4*	16.0-18.6	27.9	25.0-33.9	6.27 (<u>+</u> 0.65)
Valley-Christensen- Base	960	10.5*	9.3-11.8	20.2	16.8-28.3	4.52 (<u>+</u> 0.40)
Yolo-Selected-3	1020	4.5**	3.9-5.0	8.3	7.4-9.9	4.84 (<u>+</u> 0.33)
Yolo-Base	1060	3.5**	3.0-3.9	6.8	5.9-8.2	4.45 (<u>+</u> 0.26)

* Data were analyzed by the POLO program. Lines and slopes were different.

** The lines were different; slopes were not different.

Once we have achieved a selection plateau in a colony, and before we carry out large scale field releases, we must conduct a genetic analysis of the most resistant strain(s) to determine the mode of inheritance of the resistance. We will also need to compare life history attributes of the resistant strain with a standard strain to determine whether we need to outcross the selected strain prior to any field releases.

We are most concerned about evaluating the relationship between field rates and out laboratory doses. We conduct our selections by confining the adult parasitoids within treated plastic cups. This test method probably overestimates the toxicity of Guthion to T. pallidus because there are no untreated refuges and there could be fumigant effects in the cups. Thus, we hope to evaluate the ability of resistant and susceptible T. pallidus to survive on Guthion-treated walnut foliage. We need to determine whether our resistant strain can survive on field rates of Guthion. A subsidiary question will be to determine whether the resistant strain(s) can survive on the Guthion-treated foliage for longer intervals sooner after treatment than the susceptible strain. If the selected strain can survive field rates, or can survive on residues significantly sooner than the wild strain, then large scale implementation may be economically justified.

Because of the positive selection responses and indications of genetic variability, we want to continue the project during the next funding period to determine how high the resistances will go, and to relate this to the field rates used. If we do not obtain a resistant colony that can survive field rates significantly sooner than the susceptible strain, then we would discontinue the project at that time. We are therefore submitting a proposal for funding for one additional year.

ACKNOWLEDGEMENTS

Bill Barnett, Lonnie Hendricks, and Steve Sibbett provided information on collection sites. In addition, Wilbur Reil, Janine Hesey, Bill Krueger, and Bill Olson sent samples or colonies to us. We thank them for their help in this project.

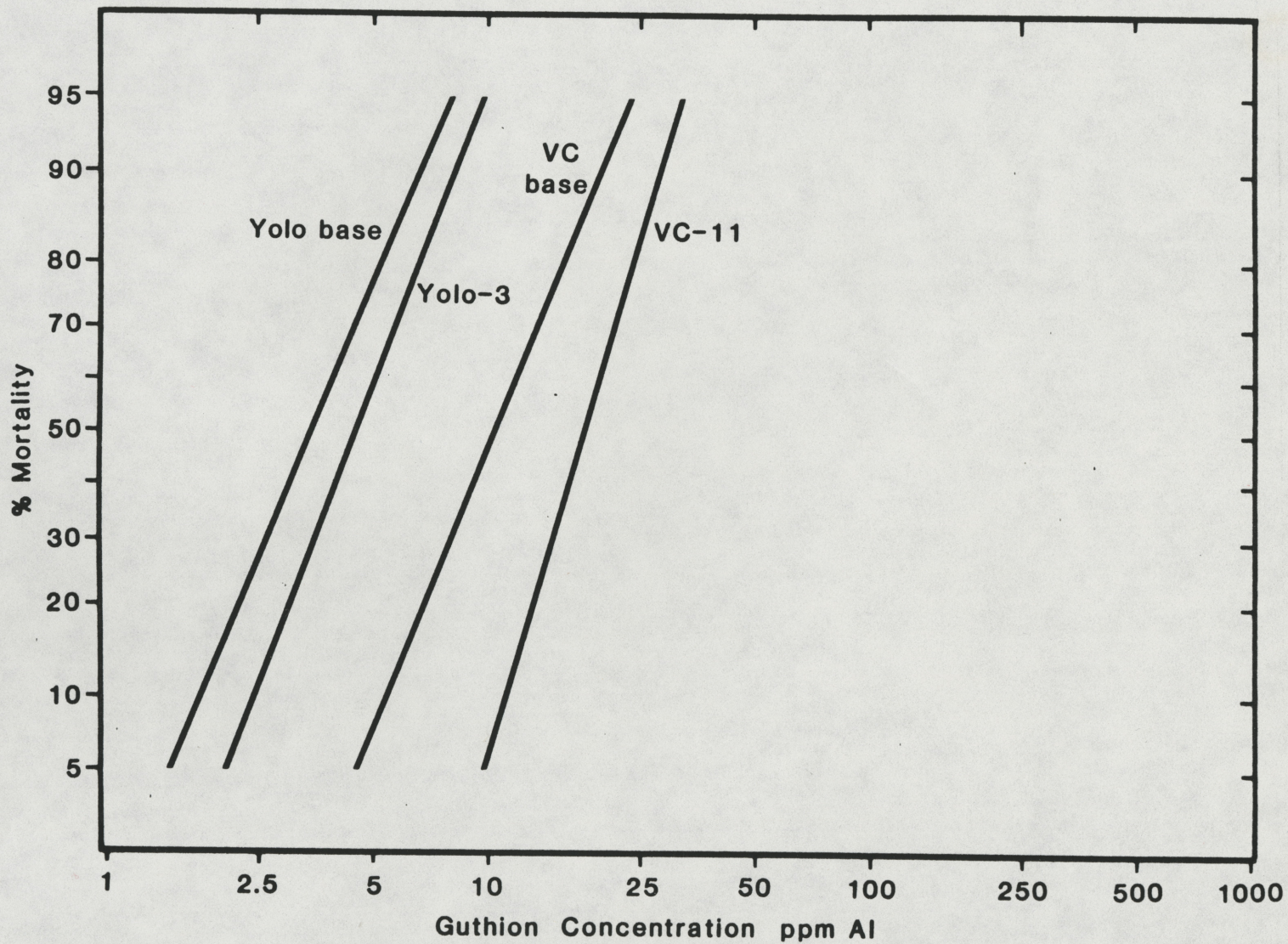


Figure 1. Concentration/mortality lines for selected and base colonies of two colonies of *T. pallidus* using a plastic cup assay with Guthion (50 WP). The Yolo and Yolo-3 colonies' lines are different, although slopes are not. Lines and slopes of the Valley-Christensen (VC) and Valley-Christensen-11 colonies are different.