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1987

1987/DAQ27L

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COTTON RESEARCH COUNCIL

FINAL REPORT

January, 1988

DAQ 27L (1986/87)

"Chemical Techniques for Determining the Age of
Adult Pests (Lepidoptera) of Cotton"

Organisation: Queensland Department of Primary Industries,
Agricultural Chemistry Branch, Biloela.

Supervisor: Mr R.M. Noble

Allocation: 1986/87 - \$ 2250

Aims: To develop a rapid chemical technique for determining
the age of adult pests (Lepidoptera) of cotton,
especially the long surviving Pectinophora scutigera
(pinkspotted bollworm).

CONTENTS

- 1. SUMMARY
- 2. REPORT
 - 2.1 Introduction
 - 2.2 Methods
 - 2.3 Results and Discussion
 - 2.4 Conclusions
 - 2.5 Recommendations for future work/dissemination
 - 2.6 Appendix (a)

1. SUMMARY

A rapid chemical method for determining the age of adults of laboratory reared Pectinophora scutigera has been developed.

Larvae were reared on artificial diet and the adults kept at 25°C until frozen for analysis.

The concentrations of four pteridines, xanthopterin, isoxanthopterin, biopterin and pterin, as well as one unidentified compound termed "conjugated xanthopterin", were determined (nanogram range) in the heads of male and female moths at ages 2, 10, 20, 30, 40 and 50 days. Analyses for isoxanthopterin in thoraces and abdomens were also made. The age of moths reared in the laboratory at 25°C is able to be determined from analysed levels of pteridines in their heads.

Pteridine concentrations in heads of moths reared at 25°C on flowers of Hibiscus spp were close to but mostly outside the range of data for those from artificial diet.

2. REPORT

2.1 Introduction

Insects of the order Lepidoptera including Heliothis spp and Pectinophora scutigera (pinkspotted bollworm) are major pests of cotton. The bollworm has been recorded mainly in central Queensland but recently has been found to have a wider distribution on hosts such as Hibiscus spp, Gossypium sturtianum and Brachychiton spp.

Because the bollworm moths are small and nocturnal and sampling for eggs is not practical the pest is difficult to detect early and control. Early results from pheromone traps gave variable results but closer consideration of components and design should improve their effectiveness. The continuing need for the pyrethroid strategy makes late season control of the bollworm difficult.

A better understanding of the population dynamics of the Lepidopterous pests is needed to assist with control. Because the adults of the bollworm can live for more than 50 days in the field a rapid means of age determination is important in these studies.

Methods for the determination of age in adult insects were reviewed by Tyndale-Biscoe¹. These are mostly laborious and often involve ovarian dissection.

Recent approaches published for Diptera by Mail et al.² and Lehane and Mail³ involve a rapid chemical technique using change in concentration with age of fluorescent eye pigments (pteridines). Attempts have also been made by Ettershank et al.⁴ to utilise the so called "age pigments" (lipofuscins).

The project aimed at developing a rapid chemical method for determining the age of adults of the Lepidopterous cotton pests especially the long living species Pectinophora scutigera.

2.2 Methods

Larval stages of P. scutigera were reared on artificial diet. Pupae were sexed and individual adults kept at 25°C until frozen for analysis.

The published method of Lehane and Mail³ for age determination in Diptera using total pteridine accumulation did not work for P. scutigera.

Individual pteridines were separated by high performance liquid chromatography (HPLC) and quantified by detection on a spectrofluorimeter.

Methods are detailed in Appendix (a)

2.3 Results and Discussion

Concentrations of the four pteridines, xanthopterin, biopterin, isoxanthopterin and pterin as well as an unidentified compound termed "conjugated xanthopterin" were determined. Table 1 lists the mean levels (ng) of pteridines in heads of adult male and female P. scutigera of different ages reared in the laboratory at 25°C. Mean concentrations of isoxanthopterin in thoraces and abdomens of adults are detailed in Table 2.

The most striking feature from Table 1 is the rapid decrease in the level of "conjugated xanthopterin" with increasing age of both male and female moths.

The concentration of xanthopterin also follows this pattern.

The concentrations of pteridines and changes with age differ for male and female in many ways. Females tend to have higher levels which may, in part reflect the different sizes of male and female but for the female these levels often increase with age while in the males there is a decrease. The data for isoxanthopterin in heads (Table 1) and in abdomens and thoraces (Table 2) illustrate this point. It is interesting to speculate about

TABLE 1

Mean concentrations (ng) of Pteridines in heads of adults of Pectinophora scutigera of different ages reared on artificial diet at 25°C

Male

Age (days)

	2	10	20	30	40	50
Xanthopterin (a)	28.7 (2.6)	15.5 (1.4)	6.9 (0.8)	5.4 (0.8)	3.5 (0.6)	4.8 (0.7)
Biopterin	4.2 (0.9)	4.6 (0.6)	3.2 (0.5)	3.5 (0.3)	3.6 (0.6)	2.9 (0.2)
Isoxanthopterin	38.3 (2.8)	33.1 (3.2)	26.2 (1.7)	24.6 (3.8)	26.1 (4.4)	22.7 (2.6)
"Conjugated (b) Xanthopterin"	92.4 (7.7)	30.7 (5.4)	10.5 (2.8)	1.5 (0.4)	3.2 (1.3)	n.d.(c)
Pterin	18.9 (1.2)	14.3 (1.1)	11.9 (0.7)	8.4 (0.8)	9.2 (0.8)	7.9 (0.9)

Female

	2	10	20	30	40	50
Xanthopterin	40.2 (6.8)	11.9 (1.4)	14.7 (1.6)	16.7 (2.4)	11.5 (1.4)	9.7 (1.8)
Biopterin	6.7 (0.9)	4.3 (0.5)	3.4 (0.4)	4.3 (0.5)	3.0 (0.2)	3.6 (0.4)
Isoxanthopterin	41.2 (2.6)	49.8 (4.7)	57.2 (4.1)	88.6 (11.3)	107 (17.1)	150 (23.6)
"Conjugated Xanthopterin"	212 (16.7)	25.0 (4.9)	11.1 (2.2)	2.5 (1.4)	1.6 (0.7)	n.d.
Pterin	20.4 (1.7)	12.3 (0.8)	11.3 (1.5)	9.5 (0.8)	8.1 (0.7)	6.9 (0.7)

a) Mean (S.E. of mean) of ten replicates except for "Conjugated Xanthopterin" - five replicates.

b) Concentration of "Conjugated Xanthopterin" expressed as equivalents (ng) of Xanthopterin.

c) n.d. not detected. Detection limit approx. 1 ng.

TABLE 2

Mean concentrations of Isoxanthopterin (ng) in thoraces and abdomens of adults of Pectinophora scutigera of different ages reared on artificial diet at 25°C

THORAX

		Age (days)				
		2	10	20	30	40
Isoxanthopterin						
Male	(a)	38.0 (4.9)	17.9 (1.4)	15.0 (2.6)	9.1 (1.1)	4.8 (1.1)
Female		54.0 (6.6)	119 (10.8)	137 (8.4)	162 (20.6)	155 (22.5)

ABDOMEN

		2	10	20	30	40
Isoxanthopterin						
Male		179 (21.1)	148 (16.4)	179 (36.7)	96 (25.0)	86 (32.5)
Female		531 (57.6)	666 (72.0)	923 (60.4)	1025 (92.0)	906 (134.0)

a) Mean (S.E. of mean) of five replicates.

the different possible biochemical roles for the pteridines in male and female.

For a method to determine age, several of the changes in pteridine levels with age are useful. Figures 1 and 2 show the changes in concentration of "conjugated xanthopterin" for male and female heads with increasing age. To complement this data a combination of pteridine concentrations is useful. Figure 3 shows the relationship with age of the product of the concentrations of isoxanthopterin and "conjugated xanthopterin" for heads of male moths. Similarly for female moths the ratio of concentrations $\frac{\text{isoxanthopterin}}{\text{"conjugated xanthopterin"}}$ (Figure 4) is useful for age determination. Thus, having determined the sex of the adult of P. scutigera reared at 25°C the age can be determined fairly precisely from these pteridine relationships.

The heads of adults of P. scutigera whose larvae had developed on flowers of Hibiscus spp were analysed for pteridines. The data (Table 3) lie close to but mostly outside the range of those for adults reared on artificial diet. The possible effect of food source for the larval stage on pteridine levels in the adult warrants further investigation.

2.4 Conclusions

A rapid chemical method has been developed to determine fairly precisely the age of adults of P. scutigera whose larval stages have been reared on artificial diet at 25°C.

While the method is rapid it does involve the use of sophisticated equipment.

Figure 1

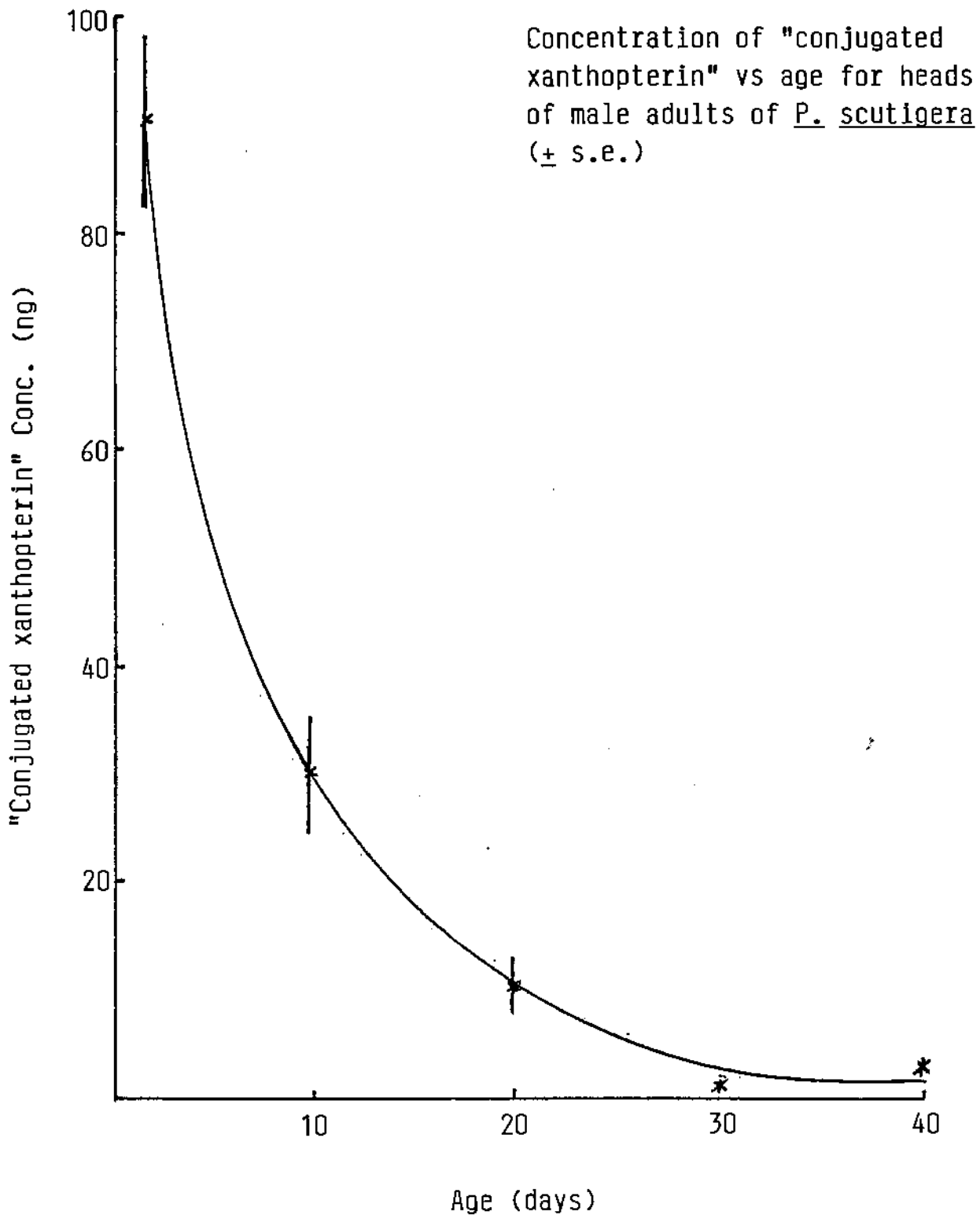


Figure 2

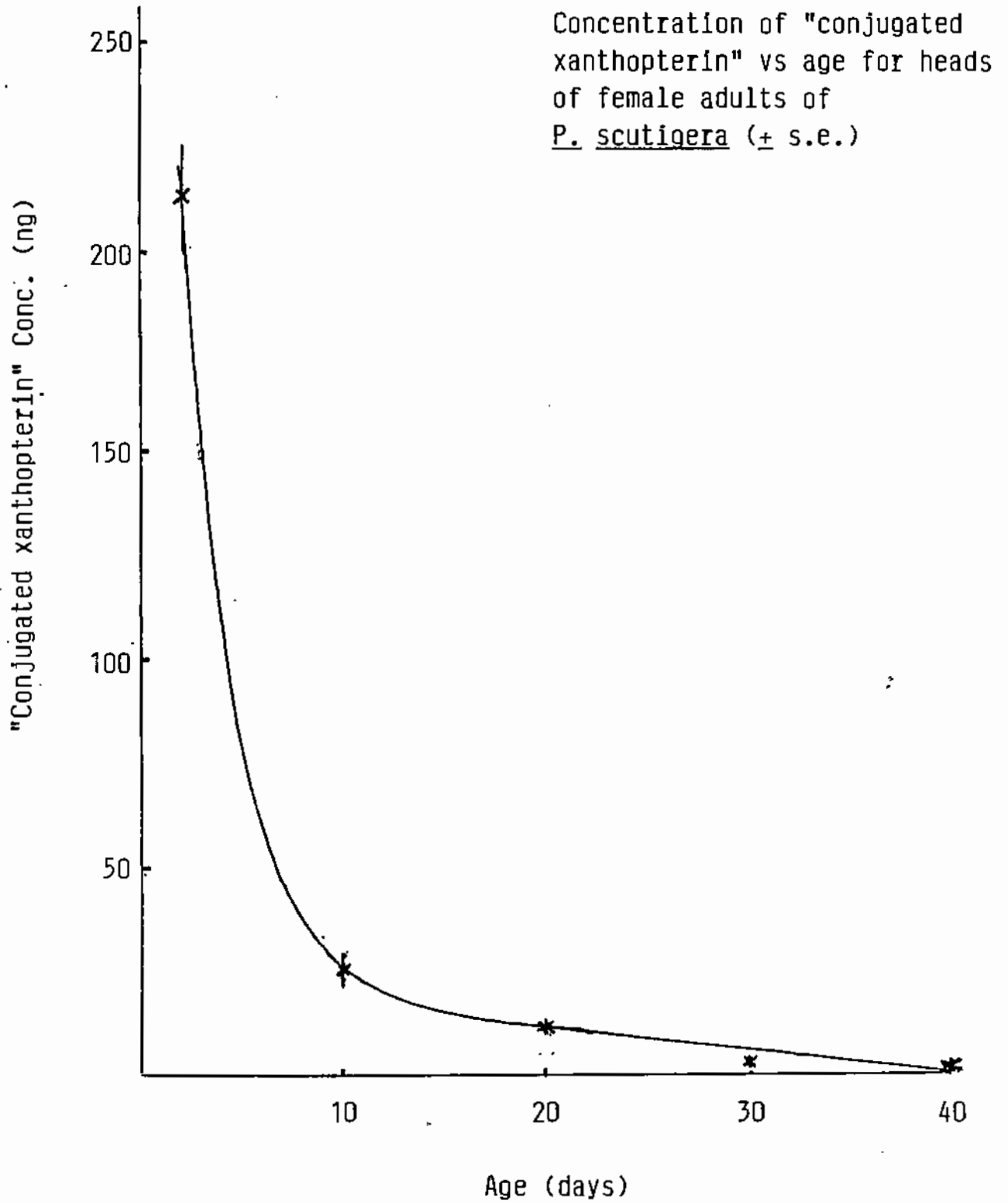


Figure 3

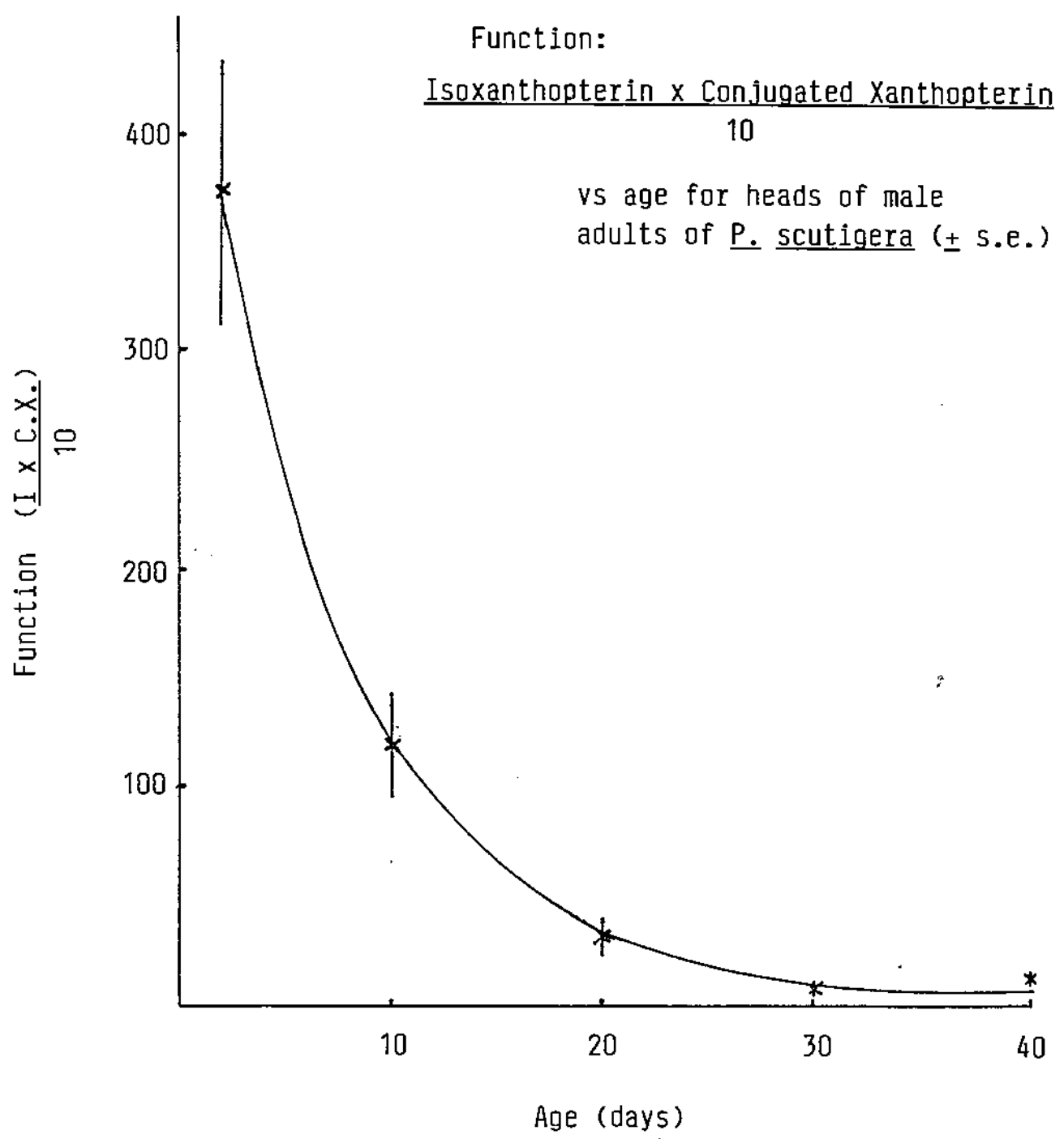


Figure 4

Function:

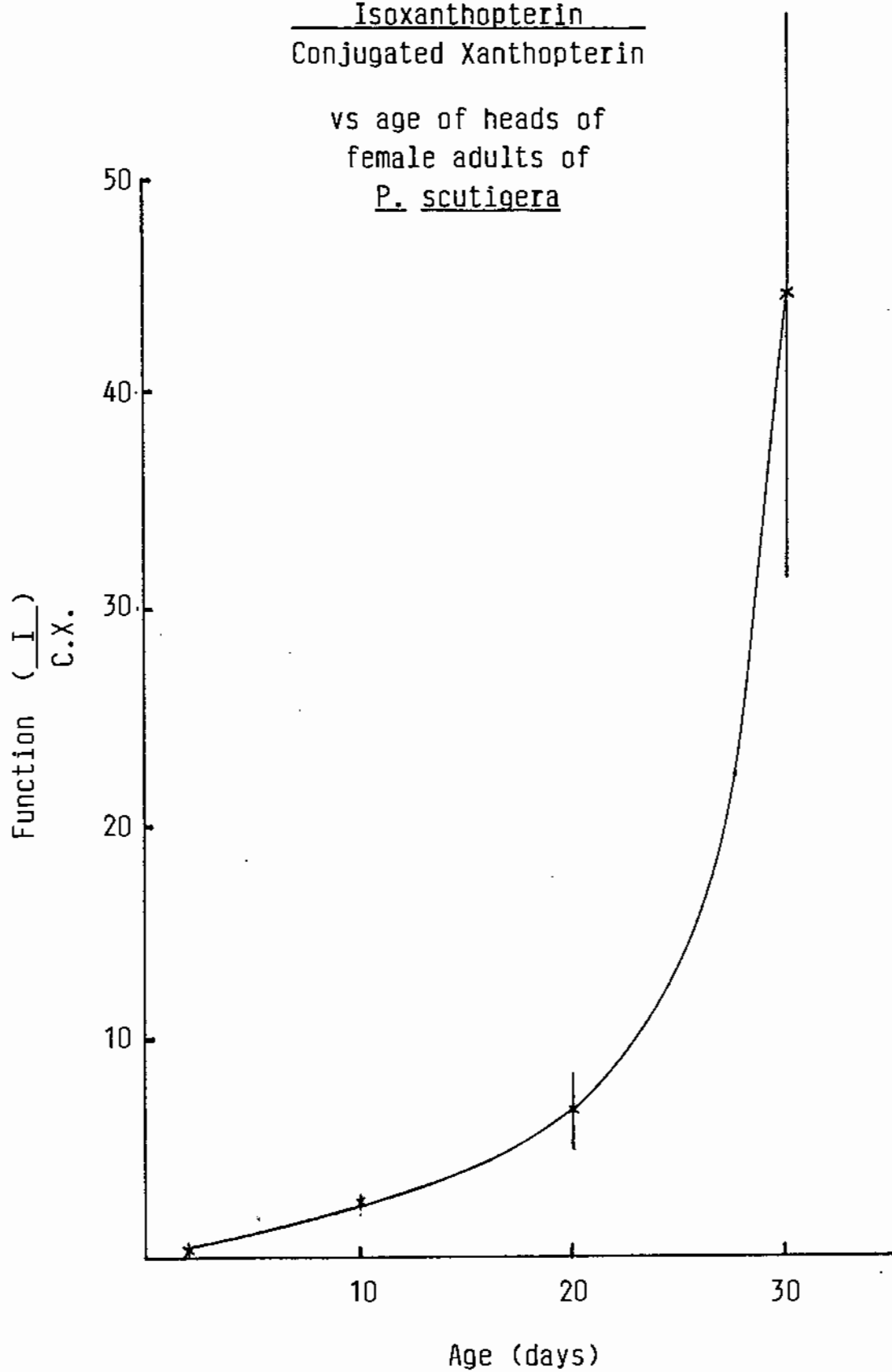
$$\frac{\text{Isoxanthopterin}}{\text{Conjugated Xanthopterin}}$$
vs age of heads of
female adults of
P. scutigera

TABLE 3

Mean concentrations (ng) of Pteridines in heads of 20 day old adults
of P. scutigera reared on flowers of Hibiscus spp at 25°C

	<u>Pteridine</u> <u>Concentration (ng)</u>	
	Male	Female
Xanthopterin	(a) 4.8 (0.6)	10.8 (1.0)
Biopterin	2.9 (0.2)	2.8 (0.2)
Isoxanthopterin	20.3 (0.9)	68.4 (7.2)
"Conjugated Xanthopterin"	6.5 (1.3)	3.8 (1.0)
Pterin	4.6 (0.3)	6.0 (0.4)

a) Mean (S.E. of mean) of five replicates.

For the method to be useful for field collected moths the effects of several parameters and especially temperature, need to be determined.

2.5 Recommendation for future work

In the current project DAQ 36L (refer separate Progress Report) the effect of temperature on pteridine levels in laboratory reared adults of P. scutigera is being investigated. The analysis of field collected adults of P. scutigera has also commenced.

Initial work has started on an organic chemical approach to the identification of host plant origins of Lepidopterous cotton pests.

Ideally, the chemical identity of "conjugated xanthopterin" should be determined. This would be a suitable project for a final year undergraduate or post graduate organic chemistry student.

Dissemination of Results

The data from DAQ 27L is at present being written up for publication. These results together with preliminary results from DAQ 36L will be discussed at the Cotton Research Workshop.

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4. Ettershank, G., MacDonnell, I. and Croft, R. (1983) - The accumulation of age pigment by the flesh fly Sarcophaga bullata Parker (Diptera: Sarcophagidae). Aust. J. Zool. 31, 131-138.

2.6 Appendix (a)

Methods

(i) Insect Rearing

First instar larvae emerging from eggs of P. scutigera supplied from Biloela Research Station were placed into separate compartments with sufficient artificial diet to complete the larval stages. Advanced instar larvae were placed into pupation chambers with tissue paper. Before emergence of the adult, pupae were divided into male and female. Adults of the same sex emerging on the same day were placed in plastic gauze-covered beakers with sucrose solution for food and stored at 25°C. The moths were killed at the required age by freezing and stored at -10°C until analysed.

(ii) Chemical Analysis

The required section of the frozen moth was removed under the microscope and placed in a tapered centrifuge tube (15 ml) with sand (0.1 g).

Pteridines were extracted by hand grinding with a rounded glass rod in two successive portions of 75 µl of solvent consisting of water + methanol + acetonitrile + acetic acid (90 + 10 + 0.5 + 0.01 by volume). The extractions were centrifuged, supernatants combined and if necessary syringed through a 4 µm filter.

High Performance Liquid Chromatography

Both C₈ and C₁₈ reversed-phase columns with a Perkin Elmer LS3B spectrofluorimeter as detector were used to separate and quantify individual pteridines.

The most useful combination was found to be:

solvent: water + acetonitrile + acetic acid (100 + 0.5 + 0.01
by volume)

column: 5 um, C₁₈ reversed-phase - 15 cm x 4.6 mm

flow rate: 1.2 mL min⁻¹

detection: Excitation 375 nm
E mission 460 nm

injection: 30 to 50 µL

volume

(10 to 20 ng of each pteridine)

The four pteridines, xanthopterin, isoxanthopterin, biopterin and pterin were identified in sample extracts on the basis of their relative retention volumes on the C₈ and C₁₈ columns and their fluorescent properties as determined on the L53B spectrofluorimeter.

Quantification was achieved by comparison with a mixed standard containing known amounts of the four compounds. The unknown compound "conjugated xanthopterin" was estimated as if it were xanthopterin as their fluorescent properties appear to be similar.

Most analyses were performed on heads but the isoxanthopterin content of abdomens and thoraces was also determined.

L. M. Nettle