



National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service

Services des thèses canadiennes

Ottawa, Canada
K1A 0N4

CANADIAN THESES

THÈSES CANADIENNES

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Investigations of the susceptibility of tomato
cultivars to *Scrobipalpula absoluta* (Meyrick)
(Lepidoptera: Gelechiidae)

THESIS SUBMITTED
TO THE SCHOOL OF GRADUATE STUDIES
AS PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

By Patricia Isabel Estay

at the
UNIVERSITY OF OTTAWA

© Patricia I. Estay, Ottawa, Canada, 1987.

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-36494-7



UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

Acknowledgements

I would like to thank to my supervisor, Dr J. T. Arnason for his guidance, criticism, time and assistance, which were freely and friendly given, throughout the course of this investigation. I am also grateful to Dr. Nozzolillo for her guidance during this work.

I would also like to thank to Dr. Bernard J.R. Philogène for having managed the Project of which this investigation is a part. The financial support was provided by the Canadian International Development Agency (C. I. D. A.).

Marisol Diaz, a technician working at the Aguila Sur Experimental Station, provided valuable assistance in the field and with laboratory studies in Chile. Esther Clérin did the typesetting and her help was also greatly appreciated. Robert Chénier provided much useful advice on the English and provided a French translated abstract. France Duval also helped me with French revisions. N analysis was undertaken through arrangements made by Drs. Fulcher and Lambert.

I would especially like to acknowledge and thank Antonio Serratos and Cathy Mc Dougall who were generous in providing their time and assistance. Antonio provided statistical support and friendly encouragement. Cathy carefully checked the English grammar in this manuscript.

Finally I would like to thank Mario, my husband and "Compañero", Francisca my daughter and my parents, who have provided all the time and moral support during the preparation of this thesis. It is to them that this thesis is dedicated.

Abstract

The quantitative assessment of susceptibility of Chilean tomato cultivars to *Scrobipalpa absoluta* (Meyrick) (Lepidoptera:Gelechiidae), the possible biological basis of resistance of certain cultivars to this insect, and the interaction between *S. absoluta* and the parasitoid, *Dineulophus phthorimaeae* de Santis (Hymenoptera:Eulophidae) were examined. Field and laboratory studies showed that within the commercial cultivars of tomato tested there is a considerable range of susceptibility to *S. absoluta* larvae. The development of the parasitoid *D. phthorimaeae* does not appear to be correlated with the tomato plant resistance suggesting that resistance and biocontrol by parasitoids could be successfully combined as a pest management strategy. An analysis of the possible antifeedant and phagostimulant compounds in tomato cultivars indicated that chlorogenic acid inhibits feeding by second instar larvae of *S. absoluta* when added to tomato leaf disks. However under field conditions there was only a marginal relationship between the susceptibility of varieties and the levels of chlorogenic acid in tomato plants. On the other hand, levels of sugar were found to be inversely and significantly correlated with susceptibility, despite the fact that sugars are usually considered as phagostimulants. Determination of sugar content using a colorimetric method might be an effective way to rapidly classify tomato plant varieties for differential resistance to *S. absoluta*. The possible interaction between sugars and some antifeedants in tomato plants is discussed.

— Résumé

L'évaluation quantitative de la susceptibilité des cultivars chiliens de la tomate à *Scrobipalpa absoluta* (Meyrick) (Lepidoptera: Gelechiidae), le fondement biologique possible de la résistance de certains cultivars à cet insecte, et l'interaction entre *S. absoluta* et le parasitoïde *Dineulophus phthorimaeae* de Santis (Hymenoptera: Eulophidae) sont examinés. Les études en laboratoire et sur le terrain démontrent que les cultivars commerciaux évalués varient de façon considérable dans leur susceptibilité aux attaques des larves de *S. absoluta*. Aucune corrélation n'apparaît entre le développement du parasitoïde *D. phthorimaeae* et la résistance du plant de tomate au Lépidophère, ce qui suggère que la résistance et la lutte biologique à l'aide de parasitoïdes pourraient être combinées avec succès et utilisées contre les insectes nuisibles dans un programme de lutte intégrée. Des composés chimiques retrouvés dans les plants de tomate ont été analysés afin de vérifier s'ils agissent comme phagorépresseurs ou phagostimulant pour *S. absoluta*. L'acide chlorogénique agit comme phagorépresseur pour les larves de second stade de *S. absoluta*, lorsqu'ajoutée à des disques de feuilles de tomate. Par contre, dans des conditions naturelles, il n'y a qu'une faible relation entre la susceptibilité des variétés et le niveau d'acide chlorogénique dans les plants de tomate. Par ailleurs, la teneur en sucres dont, plusieurs sont considérés comme étant phagostimulants, varie inversement et de façon significative avec la susceptibilité au Lépidophère. La détermination de la teneur en sucre dans les plants en utilisant une méthode colorimétrique pourrait être une façon efficace de classer les différentes variétés de tomates pour leur résistance à *S. absoluta*. L'interaction possible entre les sucres et les composés phagorépresseurs de la tomate est discutée.

Contents

ACKNOWLEDGEMENTS	i
ABSTRACT	ii
RÉSUMÉ	iii
Chapter I	1
Introduction	1
1.1 General Introduction	1
1.2 Literature review	4
1.2.1 <i>Scrobipalpula absoluta</i> (Meyrick) Lepidoptera: Gelechiidae . .	4
1.2.2 <i>Dineulophus phthorimaeae</i> (De Santis) (Hymenoptera: Eu-	
lophidae)	7
1.2.3 Tomato plant resistance to insects	8
Chapter II	13
Methods	13
2.1 Tomato Cultures	13
2.2 Insects Cultures	13
2.3 Field Resistance Test	15

2.4	Laboratory Resistance Test	15
2.5	Test Compounds and H.P.L.C. Analysis	16
2.6	Tomato Plant Extraction and Quantitative Analysis of Chlorogenic acid and Rutin	26
2.7	Spectroassay for Catecholic Phenolics (O-Dihydroxyphenols)	29
2.8	Determination of Total Sugar (Dubois <i>et al</i> , 1956)	29
2.9	Determination of Total Nitrogen	32
2.10	Paper Chromatography to Determine Sugar, Phenolic Compounds and Aminoacids	32
2.11	Feeding Deterrence Test	35
Chapter III		36
Results		36
3.1	Susceptibility of Tomato Cultivars under Field Conditions	36
3.1.1	Total Tomato Fruitmoth Larvae (Parasitized and Unpara- sitized) by Cultivar	36
3.1.2	Infestation of Tomato Fruitmoth Larvae by Parasitoid D. Ph- thorimaeae de Santis on Leaves of Tomato Cultivars	49
3.1.3	Number of Unparasitized Tomato Fruitmoth Larvae on Leaves	53
3.2	Susceptibility of Tomato Cultivars under Laboratory Conditions	53
3.3	Analysis of Tomato Cultivars for Chlorogenic acid and Rutin by H.P.L.C.	56
3.4	Total Concentration of Catecholic Phenolics	61
3.5	Sugar Content	61

3.6	Nitrogen Content	65
3.7	Determination of Sugar, Amino acids and Phenolic Compounds by Paper chromatography	65
3.8	Effect of Chlorogenic acid and Rutin on Feeding of <i>S. Absoluta</i> Larvae	65
3.9	Correlation Between Infestation or Consumption of Leaves by <i>S. Ab-</i> <i>soluta</i> and Levels of foliar catecholics, chlorogenic acid, rutin, sugar and nitrogen	69
3.10	Correlations Between Infestation by <i>S. absoluta</i> and Levels of Foliar Sugar	83
Chapter IV		85
Discussion		85
4.1	Degrees of susceptibility of commercial tomato cultivars to <i>S. abso-</i> <i>luta</i> (Meyrick)	85
4.2	Chlorogenic acid, rutin and total catecholic phenolic compounds in leaves of tomato cultivars and their relation with degrees of suscep- tibility to <i>S. absolutu</i> (Meyrick)	91
4.3	Relationship between sugar content of leaves of tomato cultivars and their susceptibility to <i>S. absolutu</i> (Meyrick)	94
4.4	Relationship between nitrogen content of leaves tomato cultivars and their susceptibility to <i>S. absolutu</i> (Meyrick)	97
Appendix 1A: Number total of <i>S. absolutu</i> (Meyrick)		98

Appendix 2A: Infestation of larvae by <i>Dineulophus phthorimaeae</i> De Santis	99
Appendix 3A: Correlation coefficient between parasitization and susceptibility	100
Appendix 4A: Infestation rate of <i>S. absoluta</i>	101
Appendix 1B: Consumption of leaf disk	102
Appendix 2B: Consumption of leaf disk	103
Appendix 1C: Correlation between larvae and chlorogenic acid	104
Appendix 2C: Correlation between larvae and rutin	105
Appendix 3C: Correlation between larvae and phenolics	106
Appendix 4C: Correlation between larvae and nitrogen	107

List of Figures

1	Structure and absorbance spectrum of chlorogenic acid	18
2	Structure and absorbance spectrum of rutin	20
3	Standard curve for chlorogenic acid using High Pressure Liquid Chromatography	22

4	Standard curve for rutin using High Pressure Liquid Chromatography	24
5	H.P.L.C. chromatogram of tomato extract using an ultrasphere . . .	27
6	Calibration curve of chlorogenic acid	30
7	Standard curve for glucose	33
8	Total tomato fruitmoth larvae	39
9	Distribution of total and parasitized populations of tomato fruitmoth larvae	42
10	Distribution of the total and unparasitized population of tomato fruitmoth larvae	44
11	Distribution of the total and unparasitized population of tomato fruitmoth larvae	46
12	Relationship between percentage of parasitization/leaf and mean to- tal number of larvae/leaf	51
13	Consumption (%) of tomato leaf disk by 4 larval stages	58
14	<i>S. absoluta</i> (Meyrick) consumption rate	67
15	<i>S. absoluta</i> (Meyrick) consumption rate as percent of control	70
16	Relationship between infestation of tomato fruitmoth larvae	72
17	Relationship between infestation of tomato fruitmoth	74
18	Relationship between infestation of tomato fruitmoth	77
19	Relationship between consumption of tomato leaf disks by first and second larval instar of <i>S. absoluta</i> and chlorogenic acid and rutin . .	79
20	Relationship between consumption of tomato leaf disks by third and fourth larval of <i>S. absoluta</i> and chlorogenic acid and rutin	81

List of Tables

1	Tomato cultivars and land race characterized according to plant characteristics and resistance to fungal disease	14
2	Characterization of different larval instars of <i>S. absoluta</i> (Meyrick)	17
3	Total number of <i>S. absoluta</i> (Meyrick) larvae on tomato varieties during summer of 1984 and 1986 in the field	38
4	Total number of <i>S. absoluta</i> (Meyrick) larvae on tomato varieties between 71 and 100 days of age during summers of 1984 and 1986 in the field	48
5	Infestation of tomato fruitmoth larvae by parasitoid <i>D. phthorimaeae</i> de Santis	50
6	Total unparasitized larvae of <i>S. absoluta</i> (Meyrick) on tomato varieties during summer of 1984 and 1986 in the field	54
7	Consumption of tomato leaf disks by first and second larval instar. .	55
8	Consumption of tomato leaf disks by third and fourth larval instar of <i>S. absoluta</i> (Meyrick) under laboratory conditions. 1984	57
9	Levels of chlorogenic acid in leaves of tomato cultivars	60
10	Levels of rutin in leaves of tomato cultivars	62
11	Levels of catecholic phenolics leaves of tomato cultivars	63
12	Total sugars in leaves of tomato cultivars	64
13	Content of <u>nitrogen</u> in leaves of tomato cultivars by Kjeldahl analysis	66

Chapter I

Introduction

1.1 General Introduction

Tomato *Lycopersicon esculentum* Mill. is economically the most important vegetable crop grown in Chile occupying an area upwards of 10,000 ha. annually. Tomato growing areas are mainly concentrated in the Norte Chico region, the region of Central Chile including the Santiago metropolitan region (Symposium Dept. of Vegetable Sciences Catholic University of Chile, 1984). At present this crop is generally cultivated commercially both in the field and under greenhouse conditions.

In Chile, the tomato fruitmoth *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is classified as a major pest on tomato based on its perennial occurrence and the level of damage it causes in North and Central Chile (Apablaza, 1984; Rojas, 1981; Matta and Ripa, 1981; Ripa, 1981; Quiroz, 1976; Vargas, 1970; Acuña, 1970).

Tomato fruitmoth *S. absoluta* (Meyrick) has also been found in tomato crops in Uruguay (Carballo *et al*, 1981), Argentina (Bahamondes *et al*, 1969), Peru (Razuri and Vargas, 1975; Sarmiento *et al*, 1976), Colombia (Garcia, 1984). Moraes *et al* (1982), Scardini *et al* (1983), and Lourencao *et al* (1984) also reported its presence in Brazil.

The larva of the tomato fruitmoth is an oligophagous specialist attacking solanaceous plants. It causes severe damage to leaves, stems, flowers and fruits of tomato (Herrera, 1963; Sanchez and Bravo, 1969; Vargas, 1970; Razuri and Vargas, 1975; Rojas, 1981). The damage to plants can destroy up to 88.9% of the commercial

fruit yields (Matta and Ripa, 1981; Moreira *et al*, 1981).

The traditional control method of this tomato pest in Chile is to spray with insecticide every 6-7 days during the summer season. According to Apablaza (1984), there was during the 1981/1982 and 1982/1983 growing seasons total dependency on chemicals for control of tomato fruitmoth in localities of the V and metropolitan region both for field and greenhouse grown tomatoes. Chemical methods of control concurrently carry the risk of high level of contaminants in the tomato crop, although few residue studies are available. Since the insecticides are applied close to or even during the harvest, the risk of residues is high. Another result of dependence on chemical control is that tomato fruitmoth has developed resistance to some insecticides (Rojas, 1981; Bravo, 1983; Bravo, 1984). At the same time, insecticides induce a very high mortality in populations of the natural parasitoid of tomato fruitmoth larvae.

An alternative to strict chemical control is Integrated Pest Management (I.P.M.) which includes the use of resistant tomato varieties, cultural control, natural enemies and chemical control. The Aguila Sur Experimental Station, in collaboration with Department of Biology, University of Ottawa, is attempting to establish an I.P.M. Program for control of tomato fruitmoth in Central Chile.

The majority of previous studies on the control of *S. absoluta* deal only with chemical control (Campos and Klein, 1967; Acuna, 1970; Campos, 1976; Ripa 1981; Carballo *et al*, 1981). However the presence of natural enemies of tomato fruitmoth (predators and parasites) has been reported in Chile (Vargas, 1970; Rojas, 1981; Santis, 1983; Larráin, 1986), Perú (Prada *et al*, 1974; Pacora, 1982), and Colombia (García, 1984). Presently, a biological control program for this insect is also being

developed in Instituto Colombiano de Agricultura (I.C.A.).

Basic information on this insect pest includes population dynamics in Central Chile, which was studied by light traps and sticky traps baited with virgin females (Quiroz, 1978)

With regard to cultural control, Matta and Ripa (1981) proposed early transplantation of tomato plants, which would advance the peak harvest period sufficiently so as to precede the period of peak larval abundance. Vargas (1970) also proposed the elimination of tomato stubble and species of alternate host weeds of the *Solanaceae* such as *Lycopersicon puberulum*, *Solanum nigrum* (L), *Solanum* sp. and *Datura stramonium* (L)

Tolerance or resistance of tomato varieties to *S. absoluta* is the only element of I.P.M. that has not yet been thoroughly examined. Lourencao *et al.* (1984) evaluated in a qualitative study eighty five wild and cultivated tomato accessions and determined that *Lycopersicon peruvianum* is a promising source of resistance to *Scrobipalpa absoluta*. Although it is possible to interbreed this species with commercial tomato cultivars, transfer of the genes for resistance would probably be time consuming and expensive.

The quantitative assessment of susceptibility of Chilean tomato cultivars to *S. absoluta* and the possible biological basis of resistance are examined in the present investigation. Nine Chilean commercial cultivars and one land race were selected for a field study infestation by *S. absoluta*. This selection of cultivars included early and late production varieties. Only one land race was available, and it was obtained from Central, Chile. Determinations of the number of larvae supported per leaf in field conditions were made during the 1984 and 1986 growing seasons.

Considering that an I.P.M. strategy requires compatibility of plant resistance with the third trophic level, the population of *Dineulophus phthorimaeae* de Santis, the principal parasitoid of tomato fruitmoth larvae, was also studied in relation to the tomato varieties.

Under more controlled conditions the feeding behavior of the four larval instars of *S. absoluta* was studied on leaf disks of the cultivars under study.

Secondary metabolites in plants are known for their biological activity as deterrents (or sometimes as phagostimulants) to insects and represent one very important type of resistance factor in the plant. Among the most prevalent secondary compounds in tomato foliage are the low molecular weight phenolics, chlorogenic acid and rutin, which have recently been shown as effective growth inhibitors to larvae of *Heliothis zea* in artificial diets (Elliger *et al*, 1981; Isman and Duffey, 1982). On the other hand, levels of sugar and nitrogen are reported as phagostimulants in the majority of insects currently studied. Therefore, hypothetical deterrents such as chlorogenic acid and rutin, and phagostimulants as sugar and nitrogen, were studied to determine the concentrations of these compounds in leaves of tomato cultivars and their relationship with *S. absoluta* larvae under field and laboratory conditions.

1.2 Literature review

1.2.1 *Scrobipalpula absoluta* (Meyrick) Lepidoptera: Gelechiidae

The tomato fruitmoth *Scrobipalpula absoluta* (Meyrick) is a microlepidopteran which occurs in South America (Rojas, 1965; Gates Clarke 1965; Herrera, 1963; Carballo *et al*, 1981; Bahamondes and Mallea, 1969; Garcia, 1984; Lourencao *et al*, 1984) and

was originally described as *Phthorimaea absoluta* by Meyrick(1917) from Perú. Povolny (1967), included this insect in the genus *Scrobipalpa*. Rojas(1965) reported its presence for the first time in Chile, but acknowledged that tomato fruitmoth larvae has probably been attacking tomato plants in Chile since 1955. At present this insect has not been reported as a pest in North America, however, Willie(1952), cited in Vargas, 1970) recognized that *S. absoluta* (Meyr.) is very similar to the tomato pinworm, *Keiferia lycopersicella* Walsingham (Lepidoptera: Gelechiidae) in shape, colour and host preference. Presently tomato pinworm is the most important tomato pest in Florida. (Pohronezny *et al*, 1986).

S. absoluta is a multivoltine insect having three to eight generations per year depending on the environmental conditions. Seven to eight may occur throughout the year under Azapa's conditions (Northern Chile), with mean temperatures between 14.4 to 22.9 C during the year (Vargas,197 In Central Chile infestations can begin in early spring.Under temperature conditions higher than 12 C, *S. absoluta* can attain three to five generations in a year (Quiroz, 1976; Rojas, 1981). Female moths oviposit throughout the day, but are preferably nocturnal. Eggs may be laid on any part of the plant, but are more commonly found on both sides of the leaves. Eggs are normally laid singly, and a female may produce a variable mean number of eggs between 52 to 237 under laboratory conditions ($25 \pm 2C$, $75 \pm 10\%RH$) (Vargas, 1970; Razuri and Vargas, 1975). At first the eggs are white to light yellow , with a mean length of 0.38 mm and width 0.21 mm, and later acquire a dark coloration close to hatching. The eggs hatch in 4 to 6 days, and the larvae develop through four instars. The duration of larval development can be between 9.5 and 27 days, depending on temperature and number of generations developed in laboratory

conditions (Razuri and Vargas, 1975). Mean duration of larval development is 27 days under constant temperature of 14.5 (Quiroz, 1976). The third generation of *S. absoluta* reared under laboratory conditions of $24 \pm 1C$ and $65 \pm 10\%$ R.H. will have a period of larval development shorter than the two previous generations lasting, 9.5 days (Razuri and Vargas, 1975). The larvae have a cylindrical body with the head flattened dorsotransversely. Newly emerged larvae have a length 0.84 mm, and by fourth larval instar reach a maximum length of 7.7 mm. The first larval instar is white, but a dark green head develops in the other instars, as a result of their feeding. The mean head capsule width is a measure utilized to differentiate each larval instar. The mean head capsule width of first instar larvae is about 0.15 mm, 0.253 mm in the second instar larvae, and 0.399 mm in the third instar, but differences are reported in the fourth instar. Vargas(1967) and Quiroz (1976) reported a mean width of 0.834 mm, and Razuri and Vargas(1975) 0.550 mm. Chrysalids pupate either on the leaves or on the soil. The chrysalid's mean length is 4.35 mm and duration of this stage is between 8 to 10 days. The tomato fruitmoth adult is a microlepidopteran with wing length of 10 mm and body length of 6 to 7 mm. Wings are dark gray and brown in coloration.

The larva of *S. absoluta* (Meyr.) is an oligophagous specialist attacking solanaceous plants, especially tomato and potato (Vargas, 1967; Herrera, 1963). According Vargas (1970), the pest also infests wild *Solanaceae* such as *Lycopersicon puberulum*, *Solanum nigrum*, *Solanum* sp. and occasionally *Datura stramonium* L; species which are present in Chile.

The tomato fruitmoth larvae grow in galleries or mines in the leaves, or burrow into stems, flowers and fruits. The damage to fruits can destroy up to 89% of the

yield. The larvae on seedlings can cause death because they feed on parenchyma (Vargas, 1970; Matta and Ripa, 1981; Moreira *et al*, 1981).

The tomato fruitmoth has received little attention with respect to host plant resistance. Recently, Lourencao *et al* (1984) evaluated eighty five wild and cultivated tomato accessions and determined that *Lycopersicon peruvianum* is a promising source of resistance to *S. absoluta*.

1.2.2 *Dineulophus phthorimaeae* (De Santis) (Hymenoptera: Eulophidae)

According to Santis (1983) *D. phthorimaeae* is a parasitoid of *S. absoluta* and potato tuber moth, *Gnorimoschema operculella* (Zeller). This parasitoid occurs in Chile, Argentine and Uruguay. In Central and Northern Chile, *D. phthorimaeae* is the most important parasitoid of *S. absoluta*, parasitizing under natural conditions about 40% of total *S. absoluta* larval population (Rojas, 1981). It is an ectoparasitoid of the second and third larval instar of *S. absoluta*. The female oviposits on these larvae generally in the host's dorsal region. In some cases, it is possible to find two parasitoids on one tomato fruitmoth larvae. The parasitized larvae lose their mobility and finally die by the action of this ectoparasitoid. The parasitoids pupate in the galleries built by *S. absoluta* in the tomato leaves. The pupae are black in color (Rojas, 1981; Larrain, 1986). The highest levels of parasitoid population are found in February and March (Central, Chile), when the population of *S. absoluta* is also at a maximum. Unfortunately, this also coincides with the highest application of pesticides (Rojas, 1981; Larrain, 1986). At the present time, no further information about *D. phthorimaeae* biology and its life cycle is available.

1.2.3 Tomato plant resistance to insects

It has been established that both the availability of nutrients in the plants and the effects of physical barriers and/or toxic allelochemicals are the important factors determining the resistance or susceptibility of plants to insects (Fraenkel, 1959; Whittaker and Feeny, 1971; Schoonhoven, 1982). Resistance of tomato cultivars and wild related species to several insects pest has been investigated with respect to these factors. The literature documents resistance to many insects including the tomato fruitworm, *Heliothis zea* (Barbosa, 1974; Beland and Hatchett, 1976; Cosenza and Green, 1979; Fery and Cuthbert, 1975; Fery and Cuthbert, 1974; Elliger *et al*, 1981; Duffey and Isman, 1981; Isman and Duffey 1982a and 1982b), leaf miners (*Liriomyza* spp) (Wolfenbarger, 1966), the tomato pinworm, *Keiferia lycopersicella* (Schuster, 1977; Schuster *et al*, 1979), the tobacco hornworm, *Manduca sexta* (Kennedy and Henderson, 1978), the colorado potato beetle, *Leptinotarsa decemlineata* (Shalk and Stoner, 1976), the beet armyworm, *Spodoptera exigua* (Hubner) (Broadway *et al*, 1986), as well tomato fruitmoth *S. absoluta* (Meyr.) larva (Lourencao *et al*, 1984), and other insects (Lange and Bronson, 1981). The modes of resistance in tomato have been documented as morphological characteristics, phenological development (such as flowering time, time o of fruiting, *et cetera*), physiological incompatibility, antibiosis, and plant age (Bronson *et al*, 1981).

Secondary metabolites found in the stems, leaves and fruit of tomato have shown biological activity against many insects, and represent one very important component of resistance for the plant. Several compounds that retard the larval growth of Lepidoptera, and some cases induce mortality, have been isolated from wild *Lycopersicon* such as *L. hirsutum* and *L. hirsutum* f. *glabratum* (Fery and Cuthbert,

1975; Kennedy *et al*, 1981; Williams *et al*, 1980). Others have also been isolated and identified from cultivated tomato leaves (*L. esculentum* Mill.) (Elliger *et al*, 1981). The major allelochemicals presently known to be found in tomato plants are phenolic compounds containing a catechol moiety (o-dihydroxy substitution) such as chlorogenic acid, rutin and a complex mixture of caffeoyl glucaric acid and its lactone. Another compound identified as an allelochemical in tomato plants is the steroidal glycoalkaloid α tomatine.

Chlorogenic acid and rutin are ubiquitous in higher plants. Chlorogenic acid is a hydrocinnamic acid (Figure 1) that is conjugated to a cyclohexane known as quinic acid. The presence of chlorogenic acid in tomato and *Solanaceae* was reported by Politis (1948), and its antibiotic effect toward *H. zea* has been described (Elliger *et al*, 1981; Isman and Duffey, 1982a and b). In other systems, such as the greenbug, *Schizaphis graminum*, it also acts as a feeding deterrent (Todd *et al*, 1971). However, in insects attacking other plants, it has been observed as a phagostimulant. For example Kato, 1978 reported that catechol based phenolics are essential for growth of the silkworm, *Bombyx mori*. The addition of chlorogenic acid to the diet improved larval growth.

Rutin is also a phenolic compound with a catechol moiety (o-dihydroxy substitution on the B ring). Rutin is a flavonol glycoside, in which the 3 hydroxyl group is bound to the sugar rutinose (Figure 2). This compound was isolated as the colouring matter of tomato stems (Blout, 1933) and its growth inhibiting effects on *Heliothis* species have been reported with cotton (Chan *et al*, 1978) and tomato cultivars (for *H. zea*) (Elliger *et al*, 1981; Isman and Duffey 1982a and b). However, the response with rutin is dependent on the insect species. Mc Farlane and Dustler

(1982) and Neville and Luckey (1971) reported that rutin enhances larval growth in the cricket (*Acheta domesticus*) when added to the insect's diet. Similar response to dietary concentrations of rutin have been found in the boll weevil *Anthonomus grandis* (Maxwell *et al*, 1967).

Isman and Duffey, 1982 found that chlorogenic acid and rutin in artificial diets are feeding inhibitors for second instar *H. zea* larvae. However, when *H. zea* were reared on excised tomato leaflets, they found significant differences in larval growth, but did not obtain a significant relationship between phenolic foliar content and larval growth. Therefore these authors suggested that for tomato, the antibiosis of isolated compounds or extracts in artificial diets may not always be an indicator of antibiosis in situ. In the foliage of the tomato plant, chlorogenic acid and rutin may constitute a large proportion (60%) of the total phenolic content of the tissue (Isman and Duffey, 1982). The foliar phenolic levels found by Isman and Duffey, 1982 from different tomato cultivars are within 0.2-0.6% fresh weight. Elliger *et al* (1981) determined concentrations of chlorogenic acid and rutin to be between 1.2-0.8 mg/g fresh wt. and 2.3-1.3 mg/g fresh wt. respectively in tomato cultivars. The same authors indicated that the effective dosage (ED50) to reduce the larval growth of first instar of tomato fruitmoth (*H. zea*) by 50 acid and 2.4 mg/g fresh weight for rutin.

Caffeic acid esters of a mixture of glucaric acid and lactone forms also occur in tomato leaves. Hydrolysis of these materials yields only caffeic acid and glucaric acid. These esters have also been described as inhibitory to the growth and development of the tomato fruitworm (*H. zea*), and therefore also represent one of the several resistance factors of the plant. They are present at approximately 1% of

the dry tomato leaf (cultivar Ace) (Elliger *et al*, 1981).

Elliger *et al*, 1981 suggested that the insecticidal antibiotic activity of the phenolic compounds chlorogenic acid, rutin, and caffeoyl aldaric acid is derived from the catechol moiety of the molecule. This moiety is able to complex with metal ions (e.g. *Cu*) which are essential for growth. Another possible mode of action was suggested by Isman and Duffey(1982b), based on the observations that early instars of *H. zea* are sensitive to certain phenolics whereas later instars are not. The ability of older larvae to rapidly overcome toxicosis could indicate that catechols act either by inhibition of feeding behavior (age-specific) or by limiting the availability of some essential nutrients which become decreasingly essential with larval age. Other possibilities include reduction of lysine availability (Davies *et al*, 1978) and non-specific enzyme inhibition (Hoover *et al*, 1977).

The steroidal glycoalkaloid α tomatine found in tomato plants and other Solanaceae is reported to be significantly more toxic to *H. zea* than the phenolic compounds, with an ED50 between 0.04 to 0.09% fresh weight (Elliger *et al*, 1981; Isman and Duffey, 1982a and b). Fontaine *et al*(1948) elucidated the structure of this compound which consists of an aglycone moiety, tomatidine, and a tetrasaccharide moiety consisting of two molecules of glucose and one each of galactose and xylose. α tomatine is found in the stems, leaves and fruits of tomato and has demonstrated biological activity against many microorganisms (Courtney and Lambeth, 1977; Irving *et al*, 1945; Levinson, 1976; Roddick, 1974). It has also been found to be a potent feeding deterrent to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Sinden *et al*, 1978) and to inhibit feeding in the potato leafhopper, *Empoasca fabae* (Dahlman and Hibbs, 1967). Bernays and Chapman, 1978 showed

that in *Locusta*, α tomatine is a feeding deterrent which interacts with phagostimulants, regulating the amount of food that this insect eats. Increasing concentrations of α tomatine reduce the amount of food eaten.

The primary metabolic function for glycoalkaloids in Solanaceus plants is not known. However because they are toxic and repellent to insects, several researchers have suggested that their primary function may be to protect the plant from insects and other pests (Buhr *et al*, 1958; Sinden *et al*, 1978).

Campbell and Duffey (1981) determined that the toxicity of α tomatine to *H. zea* and its ichneumonid parasitoid *Hyposoter exiguae* is alleviated by antidotal products in the foliage of tomato plants. These authors demonstrated that the toxicity of the alkaloid depends on levels of phytoesters in the plant, which inactivate α tomatine by complexing it. For this reason, the role of α tomatine as a resistance factor was not considered in the present study .

Chapter II

Methods

2.1 Tomato Cultures

The origin, characteristics and fungal resistance of 9 commercial cultivars and one land race obtained from Rengo, Central Chile are described in Table 1. These were grown under ambient conditions in the field. The day/night mean monthly temperatures were 21/18 in January; 20/17 in February and 18/15 in March. These means are approximate (+ - 1 C) and were very similar during the summers of 1984 and 1986. The same cultivars were grown under controlled conditions in the laboratory with mean day/night temperatures of 24/17 C and a 14:10 L:D photoperiod.

The seeds were germinated in peat pellets in December of 1983 and 1985 (spring-summer in Chile). Thirty days old seedlings were divided into two groups. One group was transplanted into plots; the other was planted in the field.

2.2 Insects Cultures

S. absoluta eggs and larvae were obtained from a colony established at Aguila Sur Experimental Station. Males and females of tomato fruitmoth were maintained on tomato plants in cages (0.70 x 0.60 x 0.85m) of wood and muslin. Eggs of *S. absoluta* from these plants were separated daily and incubated in a plastic box, diameter 2.5cm on tomato leaves maintained on water soaked paper towels. Eggs, all larval instars, pupae and adults were kept under ambient conditions. The mean

Table 1: Tomato cultivars and land race characterized according to plant characteristics and resistance to fungal disease

Cultivar	Origin	Plant Characters	Resistance to Fungal Disease
Cal Ace	Petoseed Chile	Vigorous determinate, late ripening for field crops	<i>Verticillium, Fusarium</i>
Ace 55 VF	Petoseed Chile	Large, determinate, for field conditions	<i>Verticillium, Fusarium</i>
Pakmor	Petoseed Chile	Compact, determinate for field crops	<i>Verticillium, Fusarium</i>
Duke	Petoseed Chile	Hybrid, determinate, vigorous. Early ripening under field conditions	<i>Alternaria, Stemphilum Verticillium, Fusarium S1 and S2</i>
Hybrid 6718	Petoseed Chile	Hybrid, determinate, vigorous, for greenhouse crops	<i>Alternaria, Verticillium and Fusarium</i>
Hybrid 7718	Petoseed Chile	Hybrid, determinate, vigorous, for greenhouse	<i>Alternaria, Verticillium and Fusarium</i>
Baron	Petoseed Chile	Hybrid determinate, vigorous, early ripening, greenhouse and field conditions	<i>Alternaria, Stemphilum Verticillium, Fusarium,</i>
Laser	Petoseed Chile	Hybrid without pedigree.	<i>Verticillium, Fusarium</i>
Condino land race	Rengo	Indeterminate, low production and very late ripening for field conditions.	Unknown pedigree.
Petoeary	Petoseed Chile	Indeterminate, high production, late ripening for field conditions	Unknown pedigree.

* Determinate varieties have a fixed, definite limit of growth (Scagel *et al*, 1984).

** Pedigree is referred to one's line of ancestors.

temperatures were 24/17 day/night, photoperiod was 14:10 L:D. The relative humidity was 70-75%.

2.3 Field Resistance Test

All of the tomatoes listed in Table 1 were grown in the field during the summer of 1984 and 1986 on loam textured soils, fertilized with nitrogen (90 kg/ha), phosphate (90 kg/ha) and potassium (90 kg/ha) following soil analysis.

Experimental plots were arranged in a randomized complete block design with 4 replicates, where each plot had 5 rows with 4 plants/row. The two outer rows of each plot were ignored to avoid edge effects.

Infestations with second instar larvae were made weekly from January 28 when tomato plants were 47 days old. Forty larvae were utilized for each treatment on the cultivars.

Determinations were made on randomized plants chosen (in each central row) of the number of live larvae, number of parasitized larvae by the ectoparasite *D. phthorimaea* de Santis and total larvae (as sum of unparasitized and parasitized larvae).

These measurements were started ten days after the first larvae were liberated and were performed on plants at 57,64,71,85,92,100,116,123 days old.

Observations were also made on the presence of adult tomato fruitmoth and the beginning of its attack.

2.4 Laboratory Resistance Test

Disks of 20 mm were cut out from detached tomato leaves (70-90 days old) with a cork borer and maintained in clear plastic containers (25 mm) with paper lids to

maintain humidity. These tomato disks were infested with three one day-old

larvae. For the other larval instars the disks were infested with a single larva.

Observations were made daily. Each larval instar was determined according to Vargas (1970) and Razuri and Vargas (1975) and corroborated with 30 larvae examined with a stereoscopic microscope different instars were established via head measurements of the larvae (Table 2).

When each larval instar was completed, larvae were transferred to new leaf disks and maintained under the same conditions. The third and fourth instar were transferred to new disks of tomato leaves twice. The laboratory resistance test was replicated four times. The disks were evaluated for feeding damage by counting the consumed area on a mm² grid.

2.5 Test Compounds and H.P.L.C. Analysis

The chemical structures and absorption spectrum of chlorogenic acid and rutin used in this experiment are given in Figure 1 and 2. Chlorogenic acid and rutin were supplied by Sigma Chemical (St Louis, Missouri). Purity was verified by H.P.L.C. using an ultrasphere octyl (C18) column 4.6 x 250 mm and isocratic elution with 0.5% aq. acetic acid: MeOH 50:50. The major peak for chlorogenic acid had a retention time of 5.4 min. and for rutin 14.8 min, on line. On line spectra were identical to published literature values (Markam, 1980). Standard curves of peak height versus concentration were linear. The standard curve of chlorogenic acid is shown in Figure 3 where $r = 0.93$, $F > 0.001$ and the line fitted is $y = 0.001x + 0.005$. Figure 4 shows the standard curve for rutin where $r = 0.98$, $F > 0.001$ and the line obtained was $y = 0.0017x + 0.0079$.

Table 2: Characterization of different larval instars of *S. absoluta* (Meyrick) grown at 25 ± 1 C and $70\pm 5\%$ R.H

Instar	Mean length (mm)	Mean head capsule width (mm)	color	other characteristics	duration of each stage (days)
First	0.84	0.16	white to light green	cylindrical body. The head is flattened dorsotransversally	2.8
Second	≤ 2.2	0.24	light		2.0
Third	≤ 3.8	0.37	light		2.1
Fourth	≤ 9.2	0.55	light green		2.5

* 30 larvae controlled

Figure 1: Structure and absorbance spectrum of chlorogenic acid from Markam, 1980

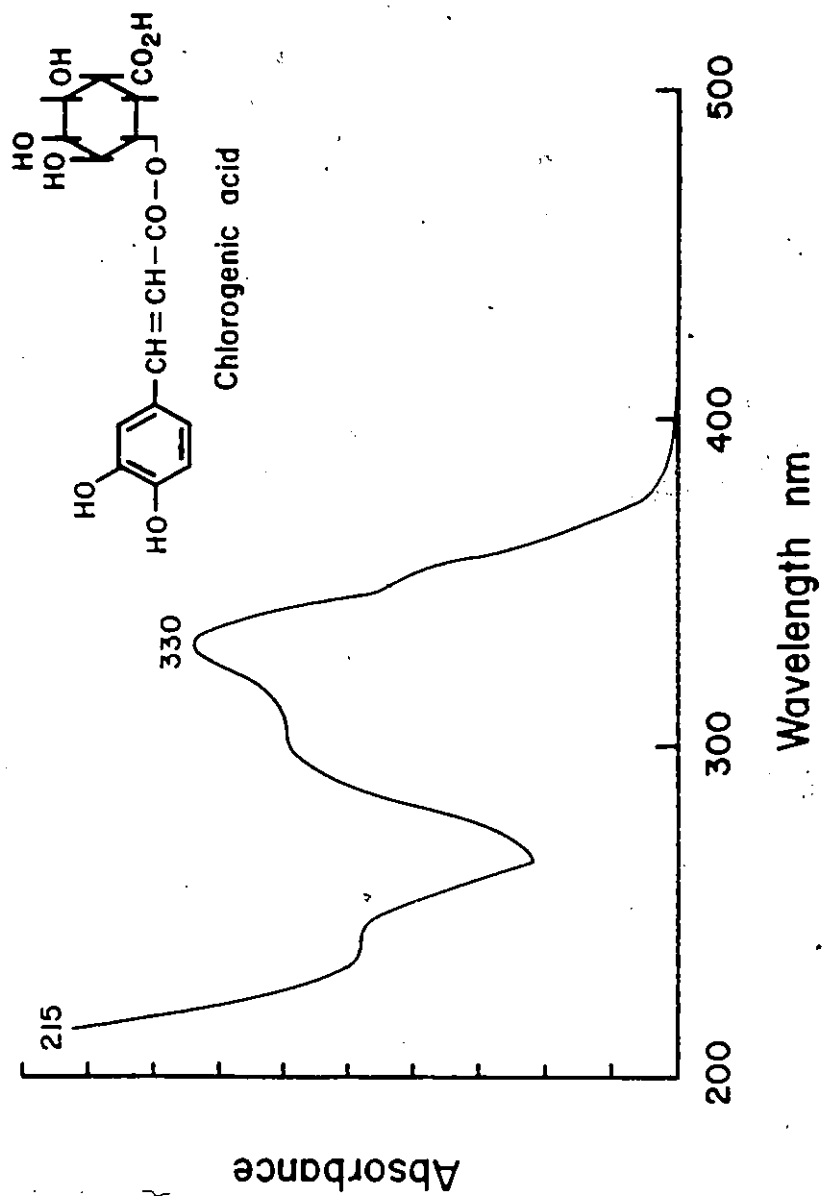


Figure 2: Structure and absorbance spectrum of rutin from Markam, 1980.

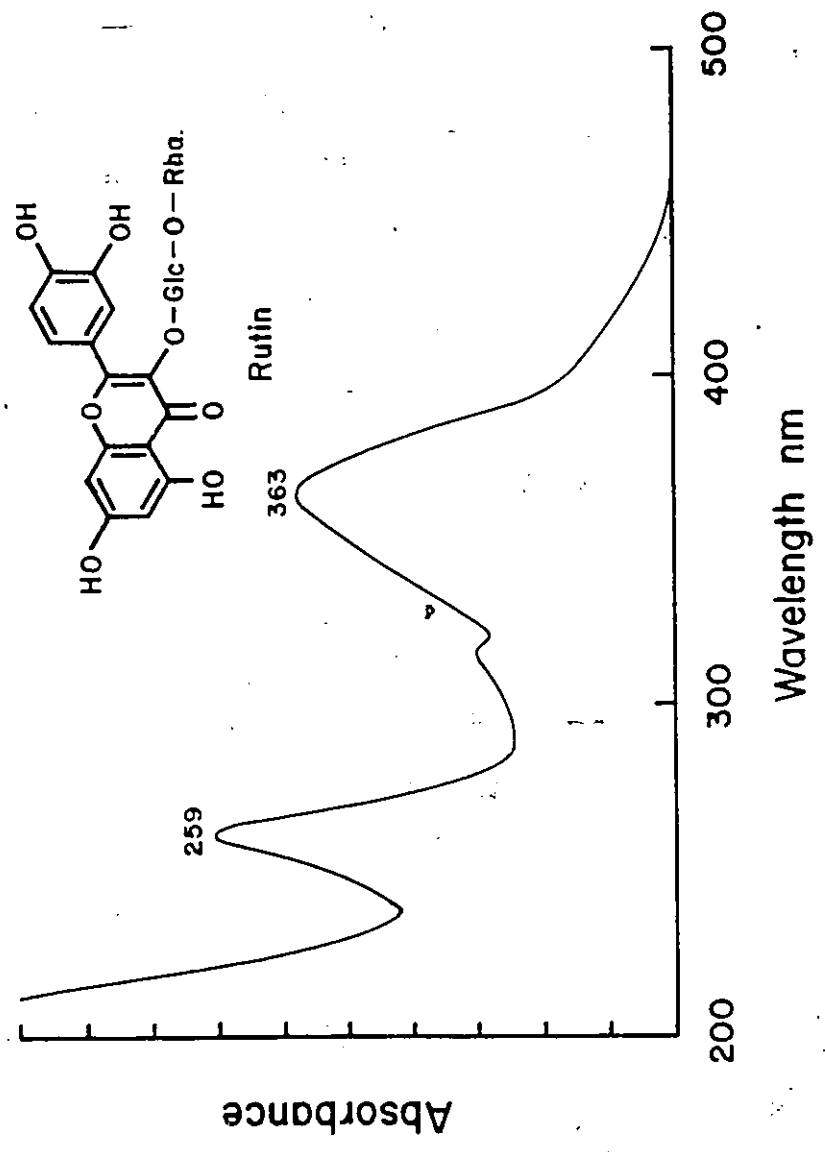


Figure 3: Standard curve for chlorogenic acid using High Pressure Liquid Chromatography, with an ultrasphere octyl column 4.6 x 250 mm and isocratic elution with 0.5% aq acetic acid MeOH (50:50) at 1 ml/min . The peak height is plotted against chlorogenic acid amount. Injection volume was 20 ul. Error bars indicate standard deviation

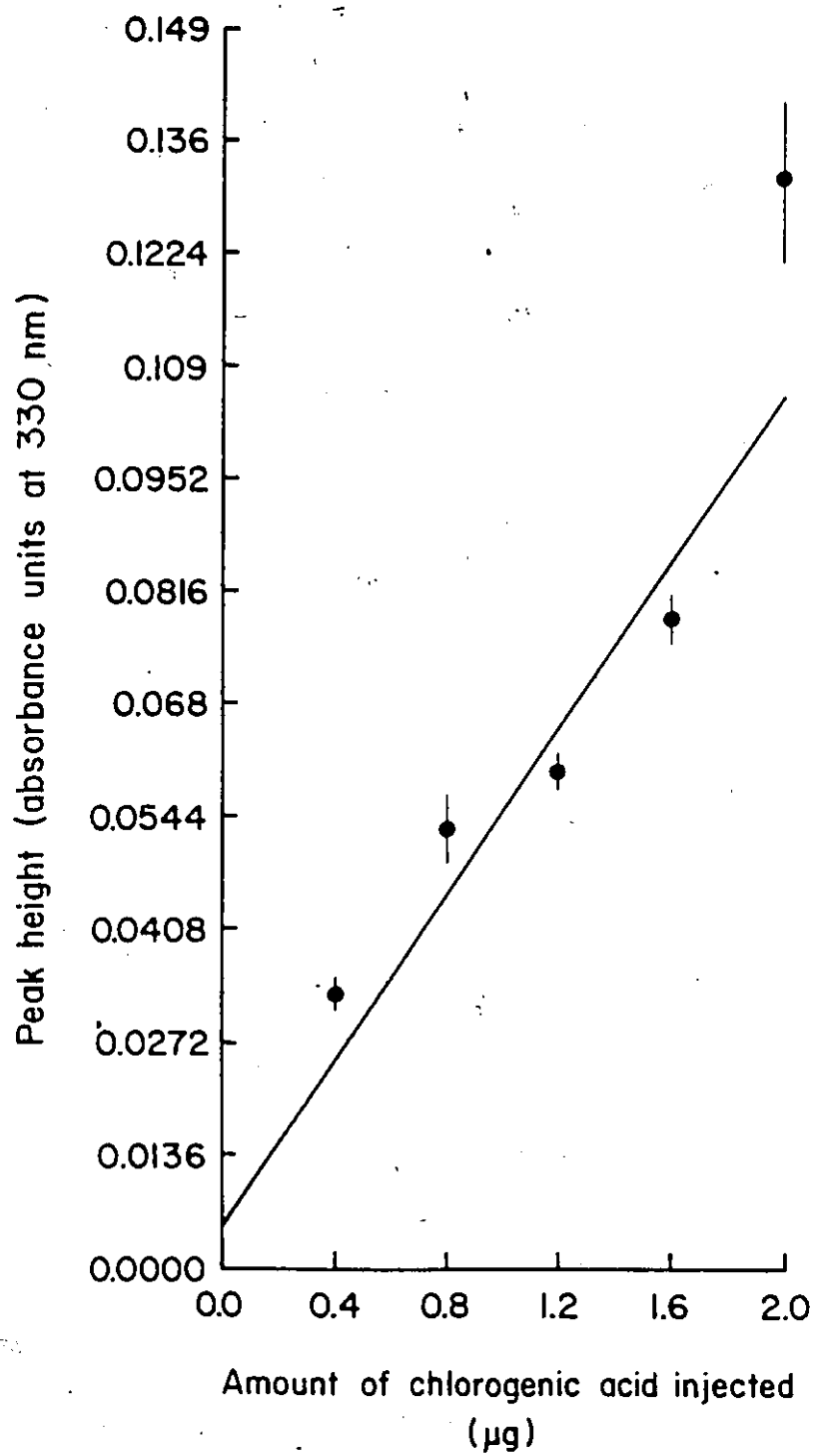
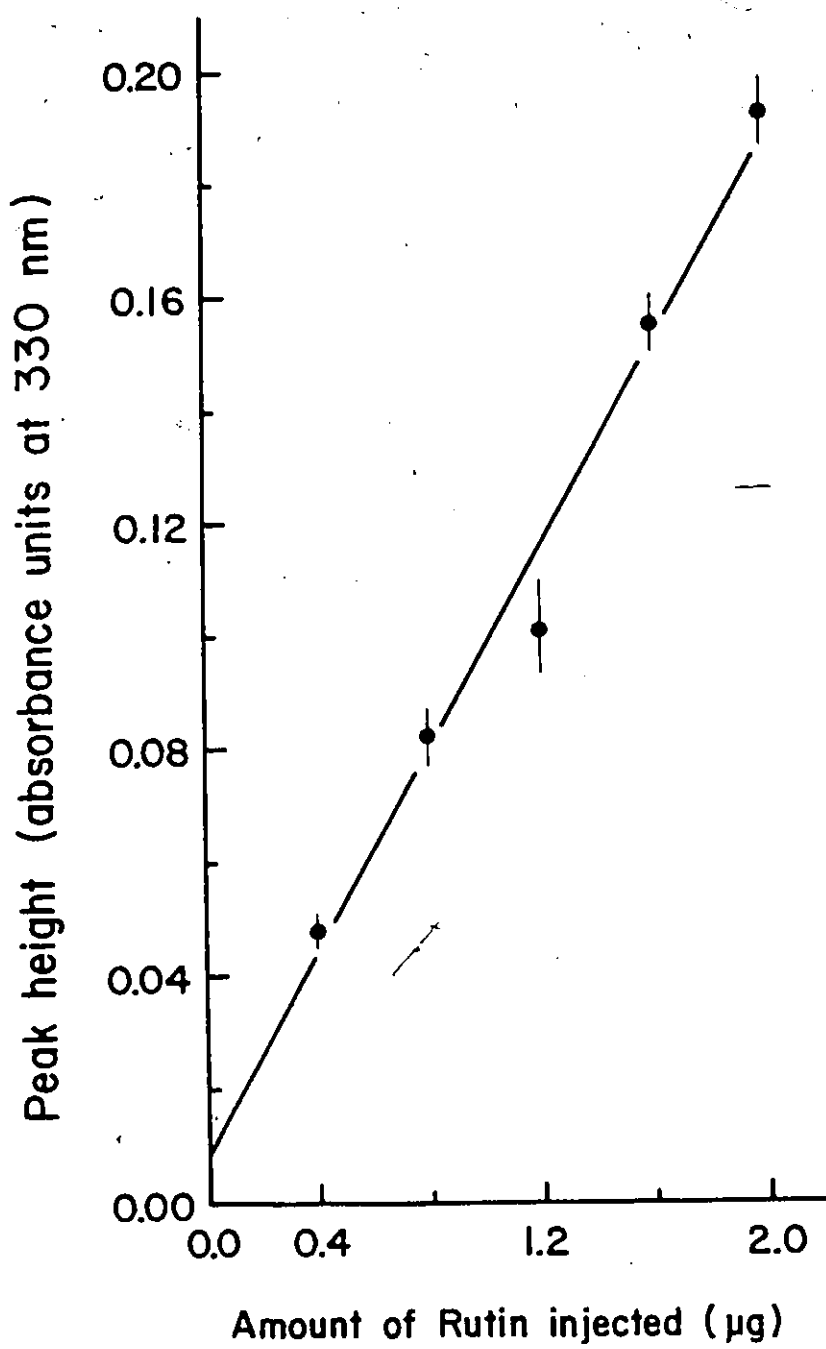


Figure 4: Standard curve for rutin using High Pressure Liquid Chromatography, with an ultrasphere octyl column 4.6 x 250 mm and isocratic elution with 0.5% as acetic acid MeOH (50:50) at 1 ml/min. The peak height is plotted against rutin amount. Injection volume was 20 ul. Error bars indicate standard deviation



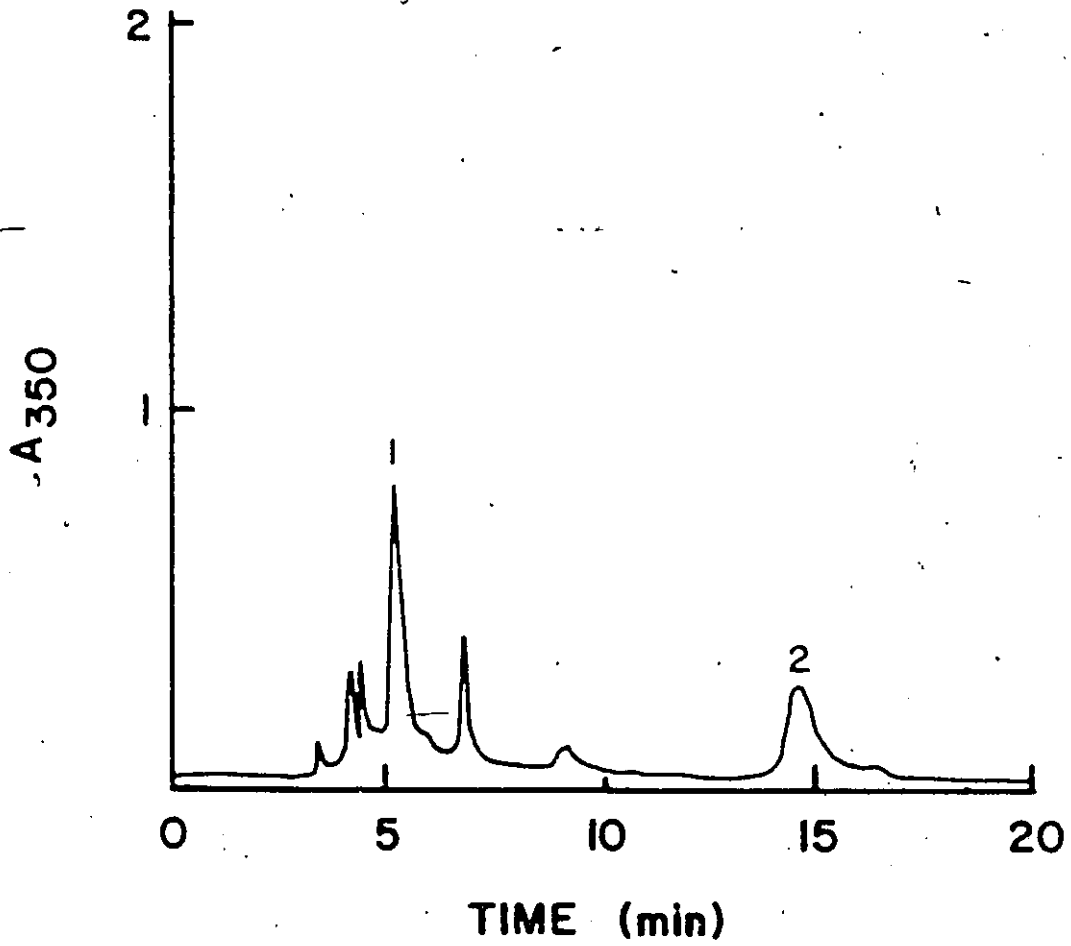
2.6 Tomato Plant Extraction and Quantitative Analysis of Chlorogenic acid and Rutin

Tomato plants were grown in a greenhouse in the Department of Biology, University of Ottawa, under a day/night temperature regime of 21/18 C. The experiment was conducted in a randomized complete block design with five replications, with each plot containing 4 plants. Young foliage of each tomato cultivar was collected at 98 days of age and the leaves were dried in an oven at 55 C overnight. % dry weight was determined. One gram of dried leaves was ground in a blender with 20 ml of CHCl₃ : MeOH : H₂O (6:4:1) containing 20 mg of quercetin. The residues were separated by filtration through a millipore filter funnel. The filtrate was shaken and combined with 10 ml of distilled water. The chloroform and methanol phases

were first diluted (with additional solvents) before the addition of water, causing the homogenate to separate into two layers: the chloroform layer containing lipids and the methanolic layer containing non lipids (Bligh and Dyer methodology, 1959).

The solutions were maintained for 24 hours in a freezer (-4 to -6 C) to separate the phases, the upper aqueous layer was analyzed by H.P.L.C. using an ultrasphere octyl column 46x250 mm isocratically eluted with 0.5% aqueous acetic acid : MeOH (50:50) as developing solvents. Rutin and chlorogenic acid were identified in the H.P.L.C. analysis by their retention time on line spectra which were identical to the standard (Figure 5). Quantification was achieved by calculation of concentration from peak height as derived from the standard curve and corroborated at each time of analysis. Final chlorogenic acid and rutin concentration were determined on a fresh weight basis.

Figure 5: H.P.L.C. chromatogram of tomato extract using an ultrasphere octyl column 4.6:250 mm and isocratic elution at 1 ml/min. with 0.5% aqueous acetic acid MeOH (50:50) . Peak 1 was identified as chlorogenic acid and peak 2 as rutin based on comparison of retention time and on line spectrum (not shown) with authentic material. Absorbance at 350 nm is plotted against retention time in min.



2

✓

2.7 Spectroassay for Catecholic Phenolics (O-Dihydroxyphenols)

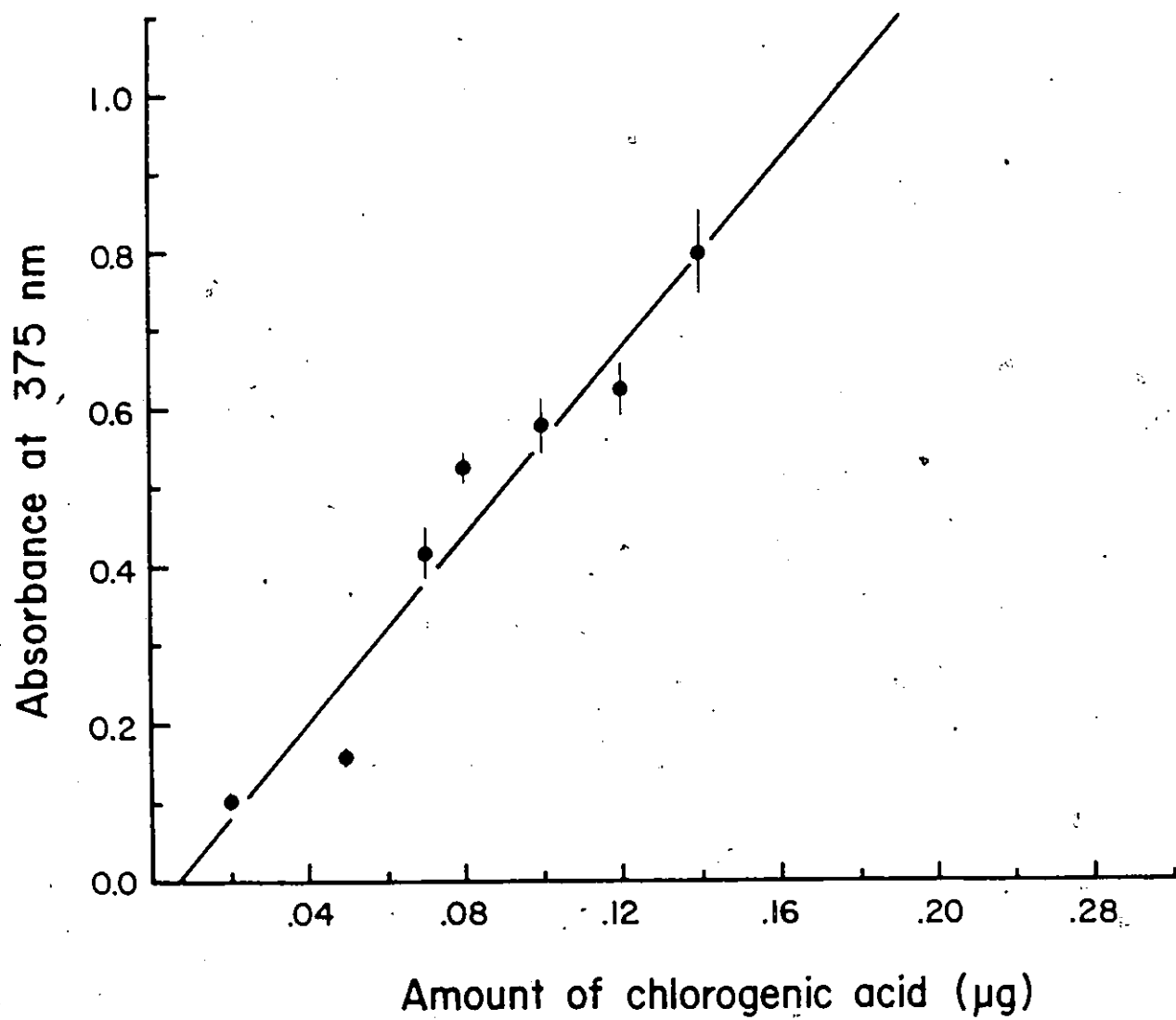
Aqueous extracts for phenolic and sugar assays were prepared from tomato leaves by macerating 0.5g dried leaves in 15 ml of EtOH : H₂O (50:50) and maintaining them overnight in a freezer (-4 to -6 C). These solutions were then filtered using a Sartorius special membrane filter (0.45 mm, 0.47N) which was rinsed with 10ml EtO : H₂O (50:50). The concentration of catechol in the extracts was determined via ammonium molybdate (Pesez and Bartos, 1974; Isman and Duffey, 1982) as follows. A saturated solution of 10% aqueous ammonium molybdate was prepared and maintained 24 hours at room temperature before use. One ml. of 0.1 N sulfuric acid was added to 4 ml of the solution of aqueous tomato extract. The reaction with molybdate in acidic medium is almost instantaneous and results in a yellow color.

The absorbance at 375 nm was read by a Coleman Junior II spectrophotometer after 10 minutes. A calibration curve using chlorogenic acid as standard was prepared, based on the range of chlorogenic acid in leaves obtained by H.P.L.C. (Figure 6), with $r = 0.97$ $F > 0.001$ and $y = 6.28$ [mg chlorogenic acid] - 0.042.

2.8 Determination of Total Sugar (Dubois *et al*, 1956)

Tomato extracts (200ul) were diluted with 1.8 ml of distilled water and 50ul of 90% phenol in water added with thorough mixing. Subsequently 5 ml of concentrated sulfuric acid was rapidly added to the mixture. The samples were maintained at room temperature for 30 minutes and the absorbance was read at 485 nm.

Figure 6: Calibration curve of chlorogenic acid as standard for determination of catecholics via ammonium molybdate method (Pesez and Bartos 1974; Isman and Duffey, 1982). The concentrations of chlorogenic acid used in this calibration curve were based on the range of chlorogenic acid in leaves obtained through H.P.L.C. Error bars indicate standard deviation.



Estimates of total sugars were derived from a standard curve for glucose (Figure 7) with $r = 0.99$ $F > 0.001$ and $y = 0.01$ [mg glucose] + 0.02. The determination was conducted in duplicate for each extract with five repetitions per cultivar.

2.9 Determination of Total Nitrogen

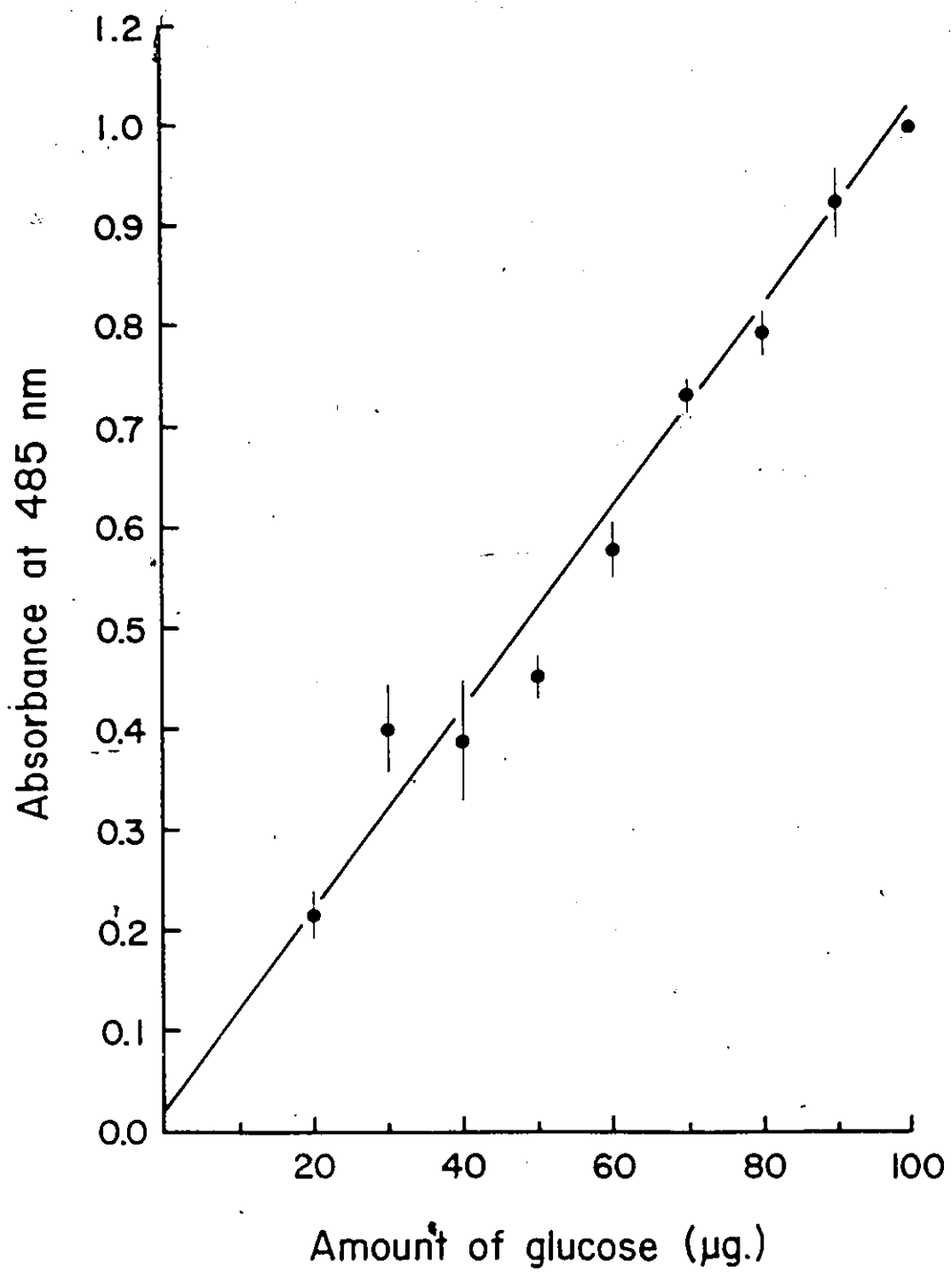
The solid residues from filtration of aqueous extracts (0.5 g dried leaves) used in the determination of catecholics and sugars were used for determination of total nitrogen without further treatment. These determination was made by Janet Gale using facilities at Ottawa Research Agriculture Canada, Ottawa, Ontario by Kjeldahl analysis

2.10 Paper Chromatography to Determine Sugar, Phenolic Compounds and Aminoacids

Chromatography of extracts to observe sugar, phenolic compounds and amino acids was performed on sheets of Whatman no.1 filter paper. n-Butanol : glacial acetic acid : water (12 : 3 : 5) was used as the developing solvent. Papers were run descendingly, overnight. Amino acids were identified by spraying ninhydrin (0.1% in n-butanol containing about 10% water) on the paper and heating for 3 minutes at 105 C. Rf values of reactive spots were determined.

A second (duplicate) paper chromatogram was used to detect sugars and phenols. Sugars were detected by spraying aniline hydrogen phthalate (aniline(930mg) phthalic acid(1.6), dissolved in water saturated n-butanol(100ml)) and were visualized permanently by heating in an oven at 105 C for 5 minutes. Phenolic compounds were identified using ammonium vapor. Chlorogenic acid was a blue color and rutin a brown color in the presence of UV light.

Figure 7: Standard curve for glucose using the method of Dubois *et al* (1956). Absorbance at 485 nm following the color reaction is plotted against glucose concentration. Error bars indicate standard deviation



2.11 Feeding Deterrence Test

The effect of chlorogenic acid and rutin on feeding behavior of second instar larvae of *S. absoluta* was tested by applying various concentrations of aqueous solutions to both surfaces of 20 mm leaf disks. The chlorogenic acid concentrations used were 0.0, 0.4, 0.8, 1.6, 3.2, 6.4 mg/g fresh wt. For rutin the concentrations used were 0.0, 0.6, 1.2, 2.4, 4.8 mg/g fresh wt.

The tomato cultivars selected for low endogenous concentrations of these compounds were "Laser" in the case of chlorogenic acid and cultivar "Baron" in the case of rutin. Control disks were treated with distilled water only. Five sibling second instar larvae of the same age were placed in a plastic box (2.5 cm diameter) with treated disks of each treatment in a randomized complete block design with four replications. The experiments were conducted in ambient laboratory conditions at 16/10 C day/night temperatures. After 48 hours the disks were evaluated for consumption by counting the consumed area on a mm grid.

Chapter III

Results

3.1 Susceptibility of Tomato Cultivars under Field Conditions

Tomato cultivars grown in the field during the summers of 1984 and 1986 showed significant differences with respect to total, parasitized and unparasitized number of tomato fruitmoth larvae.

3.1.1 Total Tomato Fruitmoth Larvae (Parasitized and Unparasitized) by Cultivar

Differences between the cultivars for the total number of tomato fruitmoth larvae (determined as the sum of unparasitized and parasitized tomato fruitmoth larvae) were remarkably consistent for the years 1984 and 1986 (Table 3; Figure 8). During both years the cultivars Baron and Cal Ace had the largest number of total larva, however, during 1984, the cultivar Laser was also among the most susceptible with no significant difference from Baron and Cal Ace. In 1986, this cultivar appeared to have significantly lower (intermediate) susceptibility than Baron and Cal Ace. The cultivars Hybrid 6718, Duke and Condino were found to have the lowest susceptibility, with the lowest number of total larvae in 1984 (without significant differences). During 1986 the cultivar Duke also had the lowest number of total larvae but there was a significant difference from the cultivars Hybrid 6718 and Condino, which exhibited a number of total larvae higher than that of Duke, but significantly lower than that of the other varieties under study. Cultivars such as Hybrid

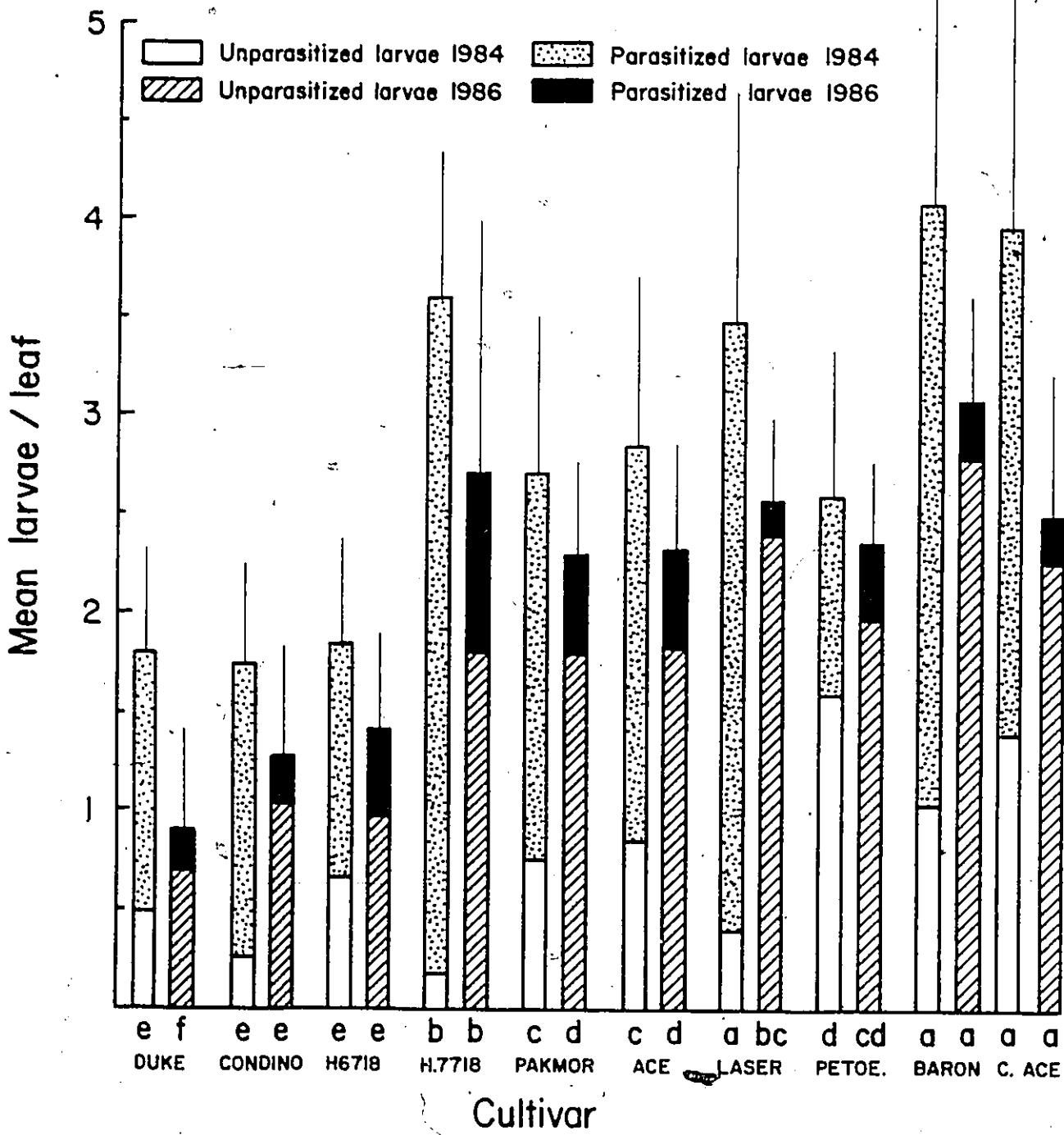
Table 3: Total number of *S. absoluta* (Meyrick) larvae on tomato varieties during summer of 1984 and 1986 in the field

		<i>Mean of total larvae (#of larvae/leaf) **</i>			
Cultivars	1984	Cultivars	1986		
Baron	4.08 a	Baron	3.08 a		
Cal Ace	3.98 a	Cal Ace	3.07 a		
Laser	3.89 a	Hybrid 7718	2.73	b	
Hybrid 7718	3.66 b	Laser	2.59	b	
Ace 55 VF	2.86 c	Petoearly	2.36	c d	
Pakmor	2.72 c	Ace 55 VF	2.33	d	
Petoearly	2.47 d	Pakmor	2.31	d	
Hybrid 6718	1.84 e	Hybrid 6718	1.42	e	
Duke	1.78 e	Condino	1.28	e	
Condino	1.73 e	Duke	0.90	f	
Mean	2.90		2.21		

* Means followed by the same letter in a column are not significantly different by Tukey's studentized range test ($P = 0.0001$)

** Based on an assessment of all cultivars placed in a randomized split plot design with 4 replicates. Each figure represents the mean number of unparasitized larvae and parasitized larvae of tomato fruitmoth on ten leaves per replicate assessed at the following plant ages: 57,64,71,85,92,100,116,123 days.

Figure 8: Total tomato fruitmoth larvae (determined as the sum of unparasitized and parasitized tomato larvae) on tomato cultivars during 1984 and 1986.— Each column represents the number of unparasitized and parasitized tomato fruitmoth larvae on 80 leaves with 4 replications per variety. Error bars represent one standard deviation and are given for total larvae only



7718, Petoeary, Ace 55 VF, Pakmor showed an intermediate susceptibility in both years.

When examining total larvae per leaf for all cultivars at different plant ages (Figure 9 and Appendix 1A), the same general trends were observed in both 1984 and 1986. The number of larvae increased to a maximum at 92 days when the plants were between flowering and fruit set. These increases in larvae from days 57 to 92 are highly significant in both years. (Detailed statistical analysis for each plant age can be seen in Appendix 1A.) Following the peak at day 92, there is a gradual decline in the number of larvae to day 123 (maturity) which is also significantly different from the peak at day 92. However, there were some differences in the pattern between the two years (1984 and 1986). For example the mean of larvae over all plant ages was 2.90 larvae/leaf in 1984 but declined significantly to 2.21 larvae/leaf in 1986. ($t = 16.8$ $df = 3.198$ $p > 0.0001$). Another difference between the years is the much more rapid increase in population in 1984 as compared to 1986, which is also evident in the statistical analysis in Appendix 1A.

The analysis of the total larvae, for individual varieties by plant age (Figure 10; Figure 11), showed that maximal infestation was also high in individual varieties in 1984 as compared to 1986. On the other hand, the susceptible varieties showed a much higher rate of increase to maximum than resistant varieties which had a much more stable population over whole the plant life cycle.

Another analysis was undertaken with only the total number of larvae between 72 and 100 days, this interval represents both the major period of infestation and the age of plants used in the laboratory study (Figure 9, Table 4). For this

Figure 9: Distribution of total and parasitized populations of tomato fruit-moth larvae at different plant ages in the field during the summers of 1984 and 1986. The beginning of flowering and fruit set are indicated on the diagram. Each point represents the mean of 100 leaves x 4 replicates

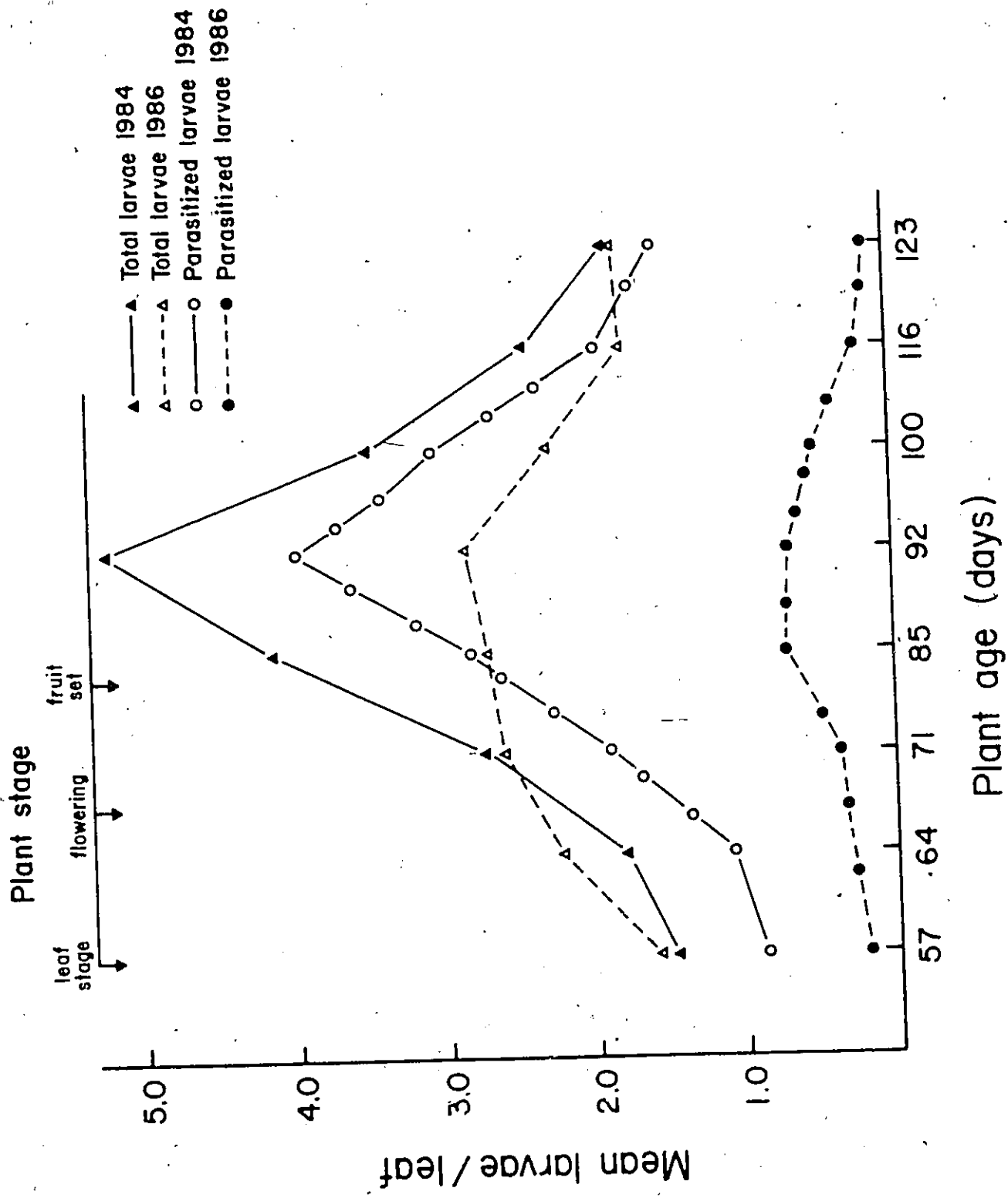


Figure 10: Distribution of the total and unparasitized population of tomato fruitmoth larvae, on each variety at different ages of plant, in the field during the summer of 1984. Each point represents the mean of larvae on 10 leaves x 4 replicate ; Error bars represent one standard deviation

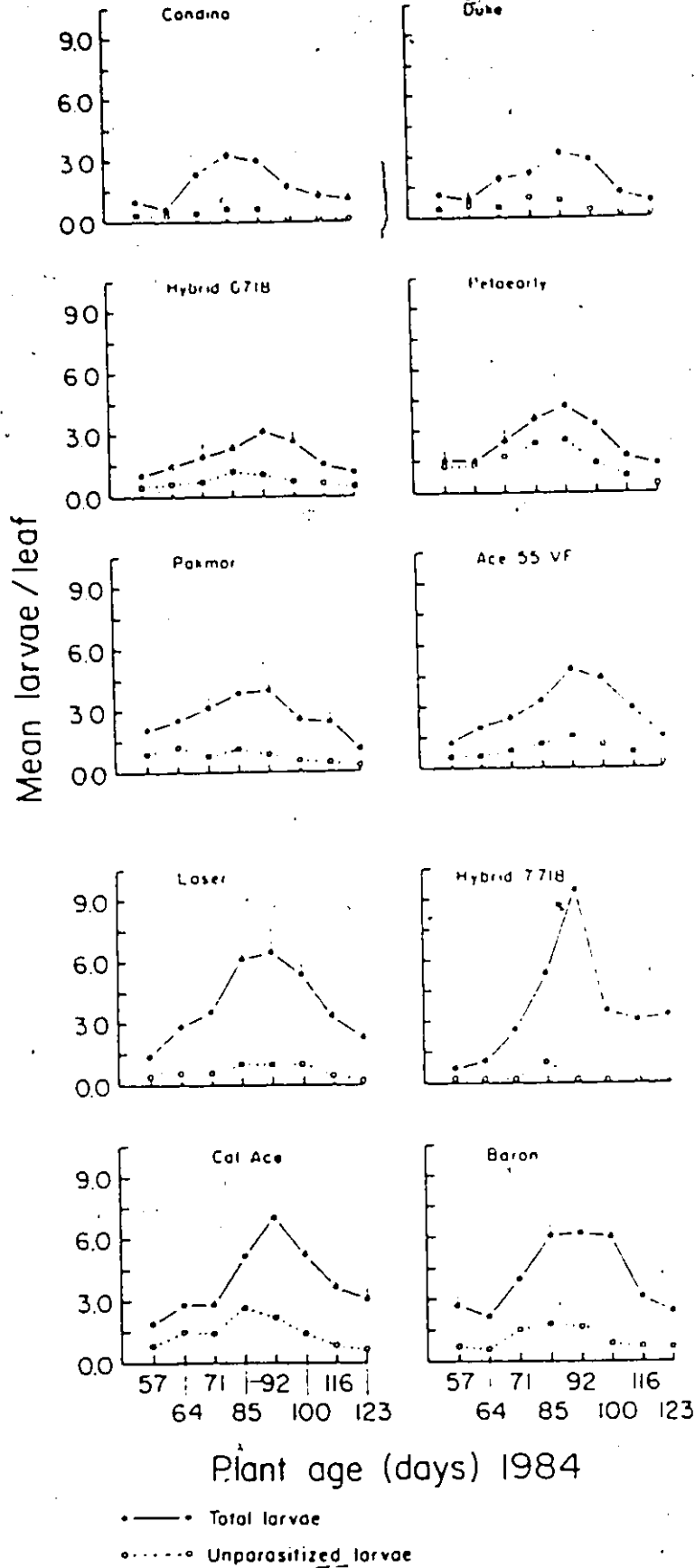
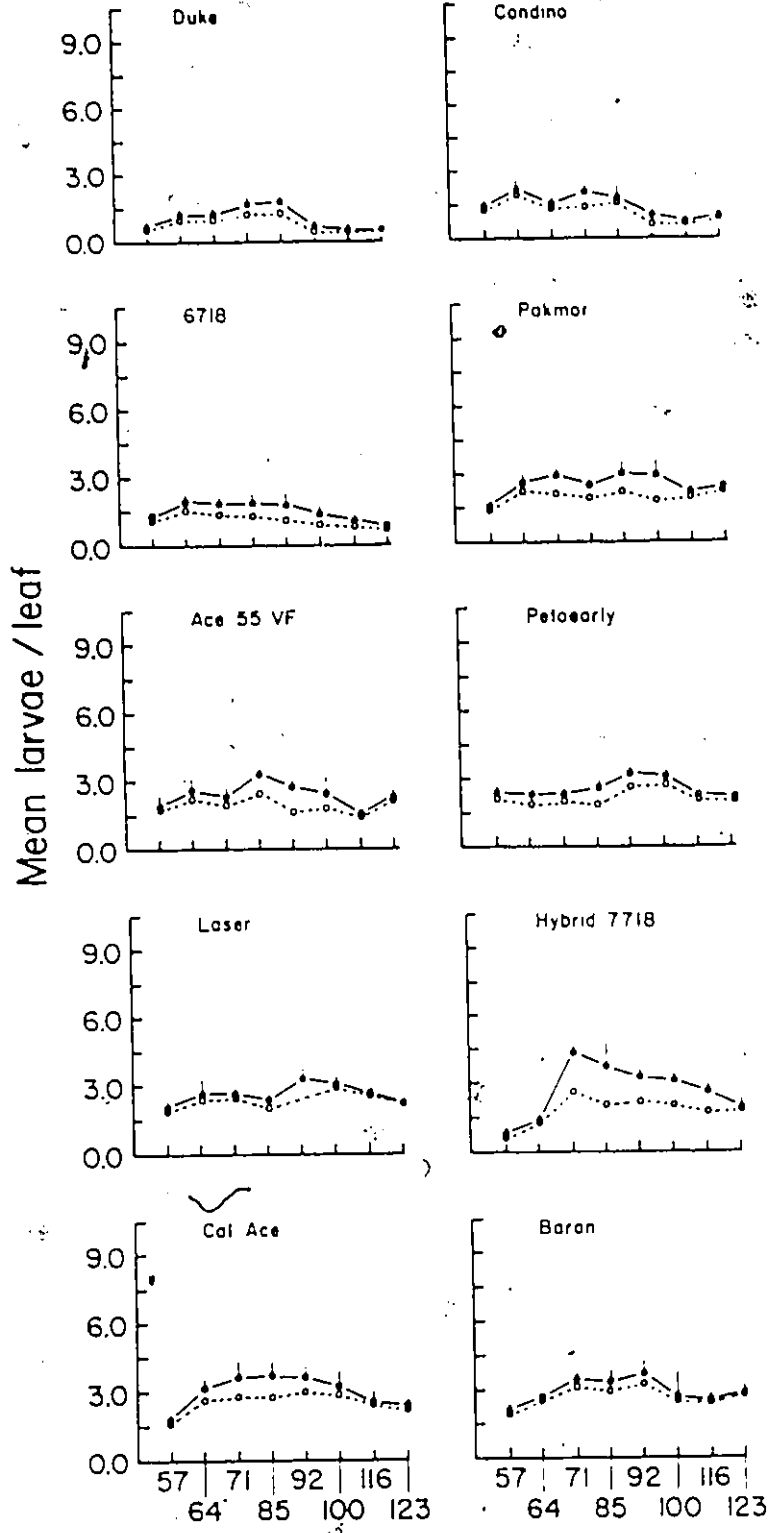


Figure 11: Distribution of the total and unparasitized populations of tomato fruitmoth larvae, on each variety at different ages of plant, in the field during the summer of 1986. Each point represents the mean of larvae on 10 leaves x 4 replicates. Error bars represent one standard deviation



Plant age (days) 1986

- Total larvae
- - - - - Unparasitized larvae

Table 4: Total number of *S. absoluta* (Meyrick) larvae on tomato varieties between 71 and 100 days of age during summers of 1984 and 1986 in the field

<i>Mean of total larvae (#of larvae/leaf) **</i>			
Cultivars	1984		Cultivars 1986
Baron	5.55	a	Hybrid 7718 3.72 a
Laser	5.36	a b	Cal Ace 3.60 a
Hybrid 7718	5.25	a b	Baron 3.46 a
Cal Ace	5.09	b	Laser 2.84 b
Ace 55 VF	3.78	c	Ace 55 VF 2.64 b
Petoearly	3.40	d	Petoearly 2.64 b
Pakmor	3.39	d	Pakmor 2.58 b
Duke	2.56	e	Hybrid 6718 1.66 c
Condino	2.50	e	Condino 1.43 c d
Hybrid 6718	2.45	e	Duke 1.22 d

* Means followed by the same letter in a column are not significantly different by Tukey's studentized range test ($P = 0.05$)

** Based on an assessment of all cultivars placed in a randomized split plot design with 4 replicates. Each figure represents the mean number of unparasitized larvae and parasitized larvae of tomato fruitmoth on ten leaves per replicate assessed at the following plant ages: 57,64,71,85,92,100,116,123 days.'

plant age range the cultivars show excellent consistency with respect to susceptibility for the whole experimental period (57 to 123 days). The cultivars Baron, Cal Ace, Hybrid 7718 and Laser showed the highest susceptibility and Duke, Condino and Hybrid 6718 had the lowest susceptibility (Table 4).

3.1.2 Infestation of Tomato Fruitmoth Larvae by Parasitoid *D. Phthorimaeae* de Santis on Leaves of Tomato Cultivars

In both years *D. phthorimaeae* de Santis parasitized larvae of tomato fruitmoth but during the summer of 1986, the number of parasitized larvae was significantly lower than the summer of 1984 (*t* - test $t = 55.9$ $df = 6.398$ $p > 0.0001$) (Table 5). The highest number of parasitized tomato fruitmoth larvae/leaf was observed on the Hybrid 7718 in both years. However the other varieties did not show total consistency between years although similar trends were evident.

Significant differences were also determined with respect to plant age (Appendix 2A) and between years (Figure 9) in relation to plant age. In 1984 and 1986 the parasitized population followed very closely and in phase of the host population. The highest number of parasitized fruitmoth larvae was observed at 92 days in both years: This was the same time as the peak of total larvae. At 57 days the lowest number of parasitized larvae was observed but was not significantly different with plants at 116 and 123 days of age.

A stepwise regression was made of the parasitization rate by variety with respect to the susceptibility (as measured by total number larvae/leaf by variety). A correlation coefficient ($r = 0.23$ $p > 0.51$) was found for 1984 (Appendix 3A, Figure 12) and ($r = -0.36$ $p > 0.31$) for 1986.

Table 5: Infestation of tomato fruitmoth larvae by parasitoid *D. phthorimaeae* de Santis on tomato cultivars grown in the field during 1984 and 1986, Central Chile.

*Mean number of tomato fruitmoth larvae parasitized/leaf ***

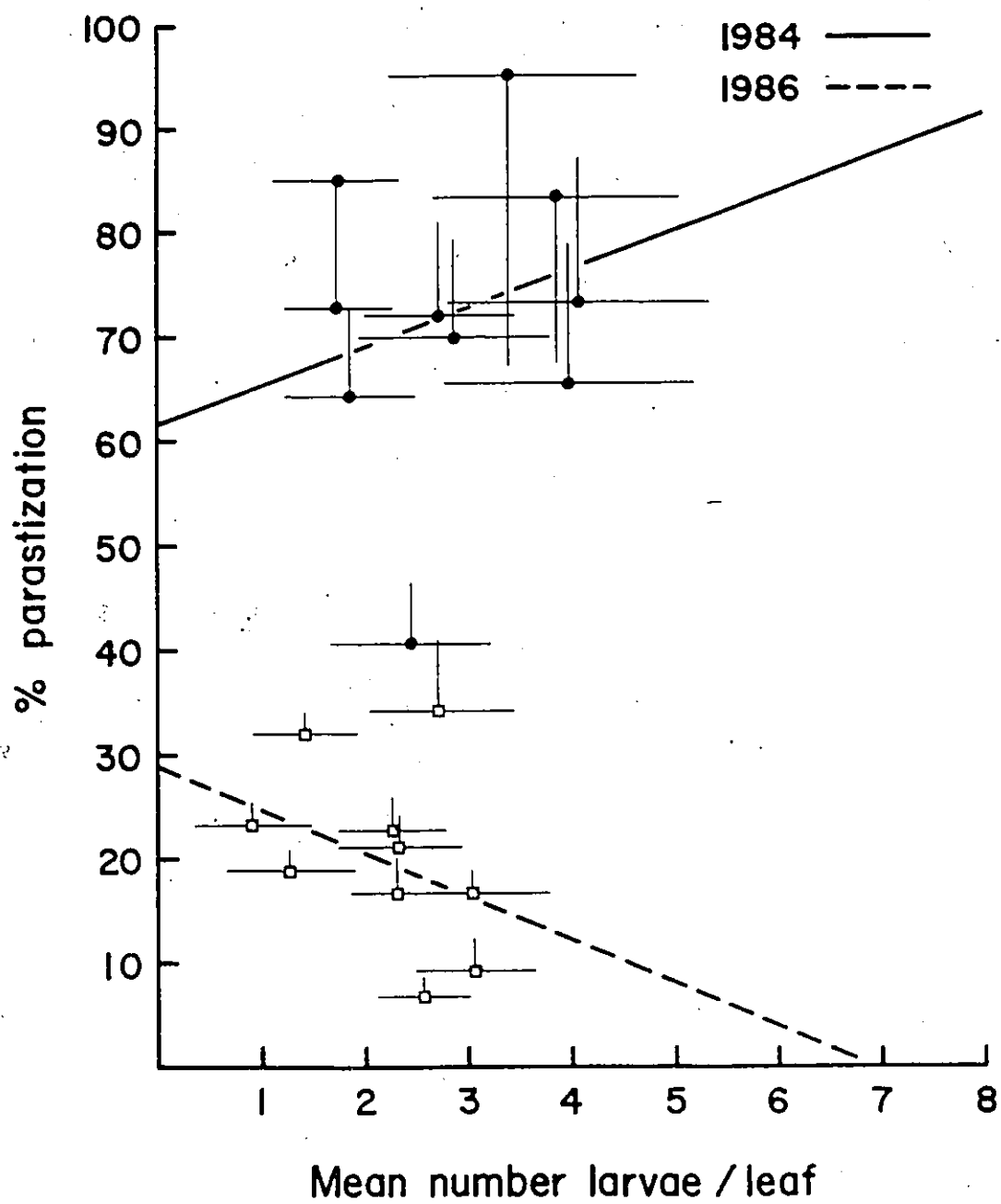
Cultivars	1984		Cultivars	1986
Hybrid 7718	3.48	a	Hybrid 7718	0.92 a
Laser	3.28	b	Cal Ace	0.52 b
Baron	3.00	c	Pakmor	0.51 b
Cal-Ace	2.60	d	Ace 55 VF	0.49 b
Ace 55 VF	2.00	e	Hybrid 6718	0.45 b
Pakmor	1.96	e	Petoearly	0.40 b c
Condino	1.48	f	Baron	0.28 c d
Duke	1.30	g	Condino	0.24 d
Hybrid 6718	1.19	g	Duke	0.21 d
Petoearly	1.01	h	Laser	0.18 d
Mean	2.13			0.42 *

* t test procedure indicates significant differences $P = 0.0001$.

** Based on an assessment of all cultivars placed in a randomized split plot design with 4 replicates. Each figure represents the mean number of parasitized larvae of tomato fruitmoth on ten leaves per replicate assessed at the following plant ages: 57,64, 71,85,92,100,116,123 days.

Means followed by the same letter in a column are not significantly different by Tukey's studentized range test ($P = 0.0001$)

Figure 12: Relationship between percentage of parasitization/leaf and mean total number of larvae/leaf in the field during 1984 and 1986. Percentage of parasitization and mean total number of larvae/leaf were obtained from the cultivars in study. Error bars represent one standard deviation



Differences between the cultivars for susceptibility to *S. absoluta* larvae were not entirely consistent but showed the same trends for both years, based on number of unparasitized larvae on leaves of tomato cultivars (Table 6).

3.1.3 Number of Unparasitized Tomato Fruitmoth Larvae on Leaves

Significant differences were observed between the years for the means of all cultivar in 1986 the population had increased with respect to 1984. (t test $t=42.2$ $df=6398$ $p > 0.0001$).

Significant differences were also found in relation to plant age and between the two years (Appendix 4A).

In both years the population showed the same tendencies observed for total and parasitized tomato fruitmoth, whereas at days 85 and 92, the population of unparasitized is significantly higher than the at other plant ages analyzed. At days 57, 116 and 123, the lowest numbers of unparasitized larvae were observed, but significant differences were found (Appendix 4A)

3.2 Susceptibility of Tomato Cultivars under Laboratory Conditions

In order to avoid the complication of parasitization in the assessment of susceptibility in varieties, laboratory tests with tomato leaf disks and fruitmoth larvae were undertaken.

During the first larval instar, the lowest damage to leaves was observed on cultivar Duke but this cultivar was not significantly different from the land race Condino (Table 7). These cultivars showed significant differences from Hybrid 7718, Pakmor and Cal Ace, which had the highest feeding rate by tomato fruitmoth.

Table 6: Total unparasitized larvae of *S. absoluta* (Meyrick) on tomato varieties during summer of 1984 and 1986 in the field

Mean of live larvae (No. of larvae/leaf) **				
Cultivars	1984		Cultivars	1986
Petoearly	1.46	a	Baron	2.81 a
Cal Ace	1.42	a	Cal Ace	2.56 b
Baron	1.05	b	Laser	2.42 b
Ace 55 VF	0.86	c	Petoearly	1.97 b
Pakmor	0.76	c d	Ace 55 VF	1.83 c
Hybrid 6718	0.65	d e	Hybrid 7718	1.82 c
Laser	0.61	e f	Pakmor	1.80 c
Duke	0.49	f	Condino	1.04 d
Condino	0.25		Hybrid 6718	0.97 d
Hybrid 7718	0.18		Duke	0.70 e
Mean	0.77			1.79*

* t test procedure indicates significant differences $P = 0.0001$.

** Based on an assessment of all cultivars place in a randomized split plot design with 4 replicates. Each figure represents the mean number of unparasitized larvae of tomato fruitmoth on ten leaves per replicate assessed at the following plant ages: 57,64, 71,85,92,100,116,123 days. Means followed by the same letter in a column are not significantly different by Tukey's studentized range test.

Table 7: Consumption of tomato leaf disks by first and second larval instar of *S. absoluta* (Meyrick) under laboratory conditions. 1984

Cultivars		(Consumption of leaf/disk (%)*	
Duke	15.2	a	
Condino	21.5	a b	
Petoerly	26.2	b c	
Ace 55 VF	26.7	b c	
Hybrid 6718	28.9	b c d	
Baron	32.0	b c d	
Hybrid Laser	32.8	b c d	
Hybrid 7718	33.2	c d	
Pakmor	35.6	c d	
Cal Ace	38.7	d	
Duke	19.9	a	
Petoerly	26.6	a b	
Condino	27.0	a b	
Hybrid 6718	34.8	b	
Hybrid 7718	45.3	c	
Hybrid Laser	46.1	c	
Baron	47.3	c	
Ace 55 VF	53.5	c d	
Cal Ace	60.2	d e	
Pakmor	64.8	e	

* Based on an assessment of all varieties placed in a randomized complete block design with 4 replicates. Each figure represents the mean percentage of consumption of three (first larval instar) and one (second instar) larvae/leaf on 10 leaf disks per replicate.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

Feeding increased with larval growth in the second and third larval instars on all cultivars (Table 7 and Table 8). The cultivar Duke and the land race Condino also showed the lowest feeding by *S. absoluta*, and in the second larval instar were significantly different from Petoeary. Results with the third instars were very similar: Duke and Condino had the lowest consumption of leaves (14.5 and 17.6 % respectively) while Cal Ace showed the highest consumption with (68.8%). The feeding of larvae on all cultivars as well as the land race decreased during the fourth larval instar (Table 8). This decrease was due to the larvae entering their prepupal phase. Nevertheless, in this stage Duke and Condino also had the lowest percentage of leaf disk consumed, with no significant differences with Hybrid 6718. Cal Ace, Pakmor, and Ace 55 VF induced the highest feeding rate in fourth instar larvae. Across all larval instars (figure 13) many cultivars were intermediate in their susceptibility, and the overlapping ranges of significance in the Duncan's test indicate a gradation in susceptibility. From the curves, it is evident that three cultivars are lowest in susceptibility (Duke, Hybrid 6718, Condino), two are intermediate (Baron Petoeary) and the rest are highly susceptible.

3.3 Analysis of Tomato Cultivars for Chlorogenic acid and Rutin by H.P.L.C.

Chlorogenic acid and rutin which are known as insect deterrents in other systems, were analyzed in all cultivars.

Table 9 shows that the highest concentration of chlorogenic acid was found in the land race Condino (3.19 mg/g fresh wt). The lowest concentrations were found in the cultivars Ace 55 VF, Pakmor, Baron and Laser (0.74 to 0.30 mg/g fresh wt).

Table 8: Consumption of tomato leaf disks by third and fourth larval instar of *S. absoluta* (Meyrick) under laboratory conditions. 1984

Cultivars		(Consumption of leaf/disk (%)) *	
Duke	14.5 a	Duke	10.2 a
Condino	17.6 a	Hybrid 6718	16.4 a b
Hybrid 6718	18.4 a b	Condino	18.8 a b c
Baron	27.7 b c	Petoearly	23.8 b c
Petoearly	35.6 c	Baron	26.6 c
Ace 55 VF	53.5 d	Hybrid Laser	28.5 c d
Hybrid Laser	58.2 d	Hybrid 7718	28.5 c d
Hybrid 7718	58.6 d	Cal Ace	30.5 d e
Pakmor	59.8 d	Ace 55 VF	33.2 d e
Cal Ace	68.8 e	Pakmor	38.3 e

* Based on an assessment of all varieties placed in a randomized complete block design with 4 replicates. Each figure represents the mean percentage of consumption of one larva/2leaf (400 mm²), with 10 leaf disks per replicate.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

Figure 13: Consumption (%) of tomato leaf disk by 4 larval stages of *S. absoluta* (Meyrick) under laboratory conditions. The numbers indicate the following cultivars.

1- Cal Ace 2- Ace 55 VF 3- Pakmor 4- Duke 5- Hybrid 6718 6- Laser 7- Baron 8- Hybrid 7718 9- Petoearly 10- Condino

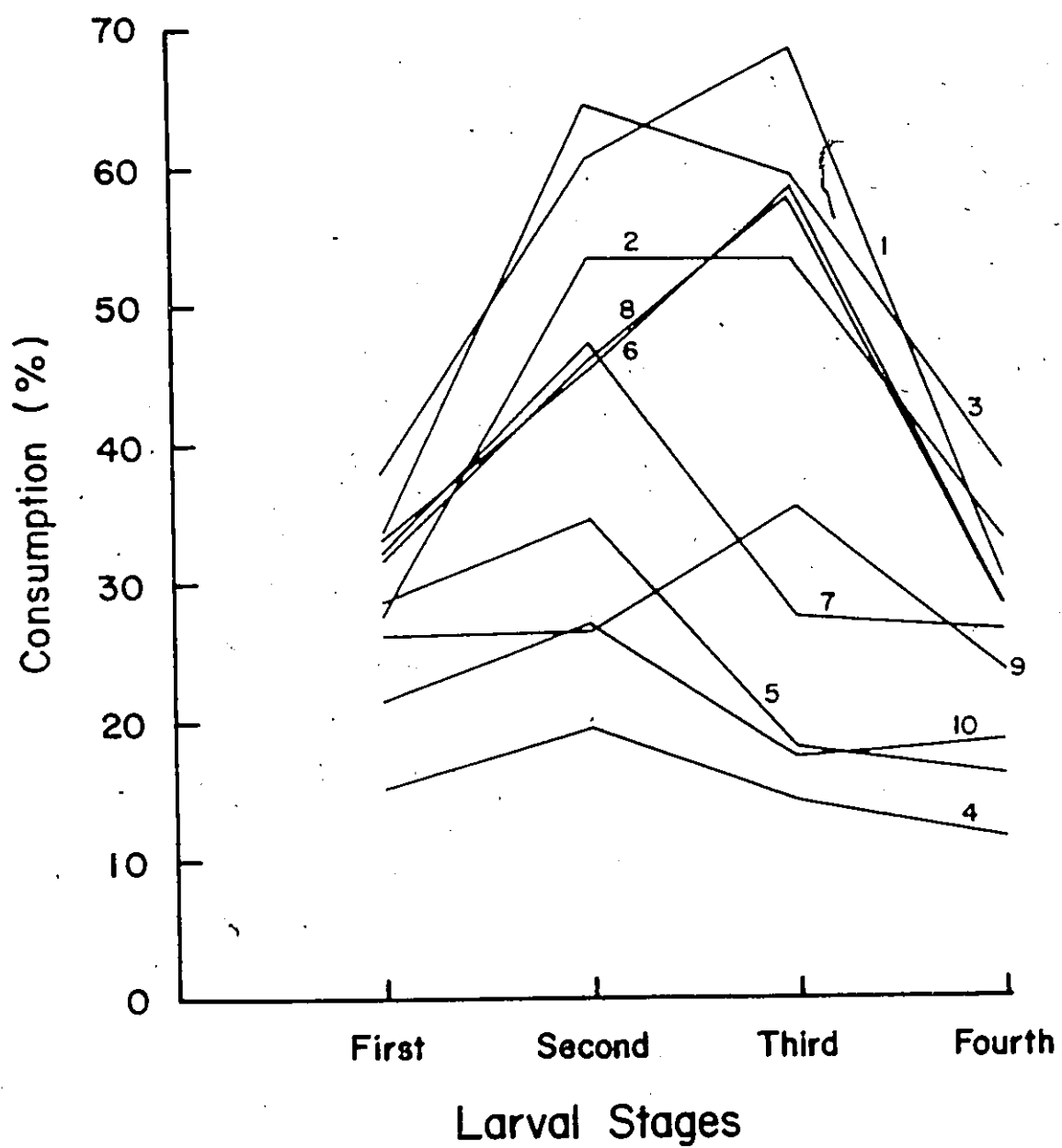


Table 9: Levels of chlorogenic acid in leaves of tomato cultivars

Cultivars	Chlorogenic acid in tomato cultivars (mg/g fresh weight)*
Condino	3.19 a
Petoearly	2.23 b
Hybrid 7718	2.21 b c
Hybrid 6718	2.00 b c d
Cal Ace	1.44 c d e
Duke	1.37 d e f g
Ace 55 VF	0.74 e f g g
Pakmor	0.59 f g g
Baron	0.40 g g
Laser	0.36 g

* Each figure represents the mean concentration of (as determined by H.P.L.C.) chlorogenic acid in four plants with five replicates at 98 days of plant age, grown under greenhouse conditions.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

It is important to note that the cultivar Duke, which showed the lowest damage to leaves under field and laboratory conditions contained only 1.37 mg/g fresh wt. This is not significantly different from the cultivar Cal Ace had the highest feeding rate by *S. absoluta* in the same test.

In the case of rutin (Table 10) the Hybrid 7718 showed a significantly higher level (2.25 mg/g fresh wt) than all other cultivars. Ace 55 VF, Duke, Pakmor, Laser and Baron had the lowest concentrations.

3.4 Total Concentration of Catecholic Phenolics

Total catechol content was found to be in the range of 2.13-8.76 mg/g fresh wt (Table 11) for all cultivars. The Hybrid 6718 had the highest catechol content but was not significantly different from cultivars Hybrid 7718, Cal Ace, Pakmor and Condino. Intermediate levels were found in the cultivars Laser, Duke Petoearly and Ace 55 VF with overlapping ranges of significance in the Duncan's test. The lowest level was found in the cultivar Baron, which also had overlapping ranges of significance in the Duncan's test with cultivars Ace 55 VF and Laser.

3.5 Sugar Content

The total sugar present in the tomato cultivars analyzed ranged between 34.7 and 8.1 mg glucose per gram foliage (fresh weight). (Table 12). The cultivars 6718, Condino, Duke and Cal Ace showed the highest levels of sugar. Ace 55 VF and Baron had the lowest levels, while intermediate levels were found in the other cultivars.

Table 10: Levels of rutin in leaves of tomato cultivars

Cultivars	Rutin in tomato cultivars (mg/g fresh weight)*		
Hybrid 7718	2.25	a	
Cal Ace	1.50	b	
Condino	1.38	b	c
Hyrid 6718	1.20	b	c
Petoearly	0.96	c	
Ace 55 VF	0.85	c	d
Duke	0.39		d
Pakmor	0.38		d
Laser	0.35		d
Baron	0.32		d

* Each figure represents the mean of concentration of (as determined by H.P.L.C.) rutin in four plants with five replicates at 98 days of plant age, grown in greenhouse conditions.

Means followed by same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

Table 11: Levels of catecholic phenolics leaves of tomato cultivars

Cultivars Free catechols in tomato cultivars as mg/g fresh weight

Hybrid 7718	7.93	a b
Cal Ace	7.53	a b
Pakmor	7.13	a b
Condino	6.92	a b c
Petoearly	5.72	b c d
Duke	5.59	b c d
Laser	4.43	c d e
Ace 55 VF	4.36	d e
Baron	2.13	e

* Each figure represents the mean of concentration of (as determined by H.P.L.C.) catecholic phenolics in 16 leaves with five replicates at 98 days of plant age, grown under greenhouse conditions.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

Table 12: Total sugars in leaves of tomato cultivars

Cultivars	Total sugar in tomato cultivars		
Hybrid 6718	34.7	a	
Condino	33.9	a	
Duke	27.6	a b	
Cal Ace	26.4	a b c	
Petoearly	22.5	b c d	
Laser	21.7	b c d	
Pakmor	19.6	b c d	
Hybrid 7718	17.6	c d	
Ace 55 VF	13.5	d e	
Baron	8.1	e	

* Each figure represents the mean concentration of sugar content(analyzed by the methodology of Dubois *et al* 1956) of 16 leaves with five replicates assessed at 98 days of plant age, grown under greenhouse conditions.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

3.6 Nitrogen Content

The results of the determination of total nitrogen are shown in Table 13. Significant differences in the content of nitrogen were found between the cultivar Baron and all the other cultivars. The Hybrid 7718 and Petoearly showed the lowest percentage of nitrogen in leaves.

3.7 Determination of Sugar, Amino acids and Phenolic Compounds by Paper chromatography

Glucose and sucrose were observed to be main the sugars present on chromatograms of tomato leaf extracts. The main phenolic compounds separated were, as expected, chlorogenic acid and rutin. Phenylalanine was the only amino acid detected in all cultivars under study by paper chromatography.

3.8 Effect of Chlorogenic acid and Rutin on Feeding of *S. Absoluta* Larvae

Significant antifeedant activity with chlorogenic acid added to leaf disks was observed at concentrations of 1.6 mg/g fresh weight and above (Appendix 1B). A regression line through the data showed a significant inverse relationship (Figure 14) $y = -27(\log C) + 68.9$, $r = -0.83$, $p = 0.05$ where y is the consumption rate as % of control and $C = [\text{mg chlorogenic acid}]$. A PC50 (protective concentration for 50% inhibition of feeding) of 2.4 mg/g wt ($\log C = 0.38$) was calculated from the regression line.

At low concentration rutin had a phagostimulant effect on larval feeding (Appendix 2B). The concentration of 0.6 mg/g wt increased the feeding by 100%, while the concentration of 4.8 mg/g wt inhibited feeding significantly.

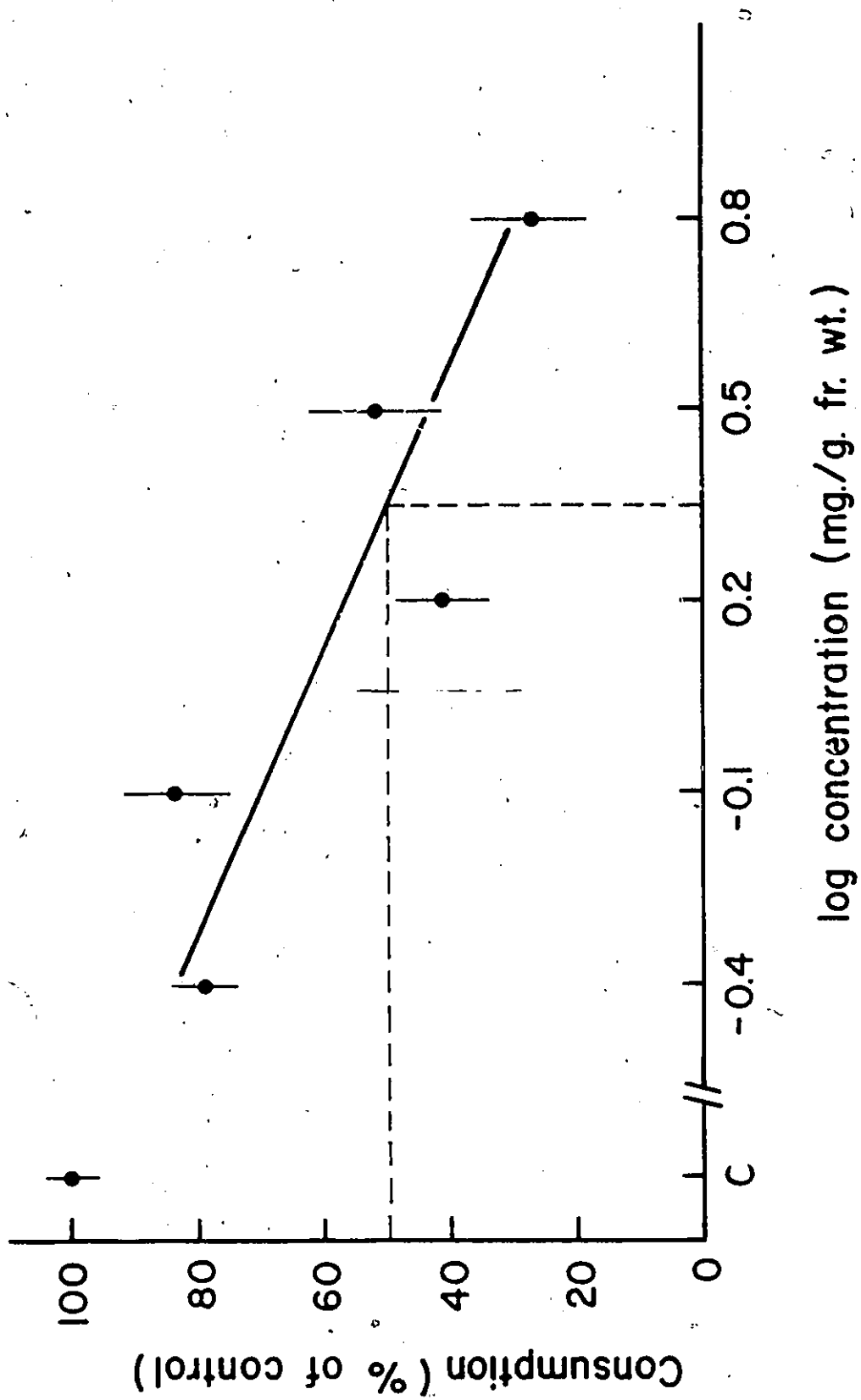
Table 13: Content of nitrogen in leaves of tomato cultivars by Kjeldahl analysis

<i>Cultivars</i>	<i>Percent nitrogen in dry tomato leaves *</i>		
Baron	5.68	a	
Ace 55 VF	4.75	b	
Pakmor	4.64	b	c
Hybrid 6718	4.15	b	c
Laser	4.00		c d
Condino	3.59		d e
Cal Ace	3.24		e
Duke	3.22		e
Hybrid 7718	2.56		f
Petoearly	2.44		f

* Based on an assessment of all cultivars placed in a randomized complete block design with five replicates. Each figure represents the percentage of nitrogen in 16 leaves per replicate assessed at 98 days of plant age grown under greenhouse conditions.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

Figure 14: *S. absoluta* (Meyrick) consumption rate as percent of control in response to different concentrations of chlorogenic acid. The dashed line indicates the concentration required to produce a 50% decrease in consumption.



The EC50 for stimulation of feeding was 1.28 mg/g wt ($\log C=0.10$) (Figure 15). There was no change in feeding at 2.28 mg/g wt ($\log C=0.35$). For inhibition of feeding the PC50 was 4.16 mg/g wt., and the regression line obtained was $y = -199.5(\log C) + 173.2$ $r = -0.991$ $p > 0.001$ where y the consumption rate as % of control and $C=[\text{mg rutin}]$.

3.9 Correlation Between Infestation or Consumption of Leaves by *S. Absoluta* and Levels of foliar catecholics, chlorogenic acid, rutin, sugar and nitrogen

The susceptibility results obtained in the field for total and unparasitized tomato fruitmoth larvae in 1984 and 1986, and the laboratory assays of leaf disks consumption by the four larval instars were analyzed in relation to the concentration of catecholic phenolics, chlorogenic acid, rutin, sugar and nitrogen. The correlation analysis revealed a significant inverse relationship between the concentration of sugar in the leaves at 98 days of age and the means of total number of tomato fruitmoths per leaf in 1984 and 1986. A regression line fitted to this data for 1984 (Figure 16) was determined through a stepwise regression procedure. $y = -0.075$ [mg glucose] + 4.61, $r = -0.67$ $p > 0.033$

The regression line fitted for 1986 data (Figure 17), also determined through stepwise regression procedure, was: $y = -0.063$ [mg glucose] + 3.63 $r = -0.70$ $p > 0.024$

When comparing the total larvae in the field and the levels of chlorogenic acid in leaves during 1984 and 1986 (Appendix 1C), an inverse relationship was also




Figure 15: *S. absoluta* (Meyrick) consumption rate as percent of control in response to different concentrations of rutin. One dashed line indicates the concentration required to produce a 50% increase in consumption. The other dashed line represents the concentration for 50% inhibition of consumption

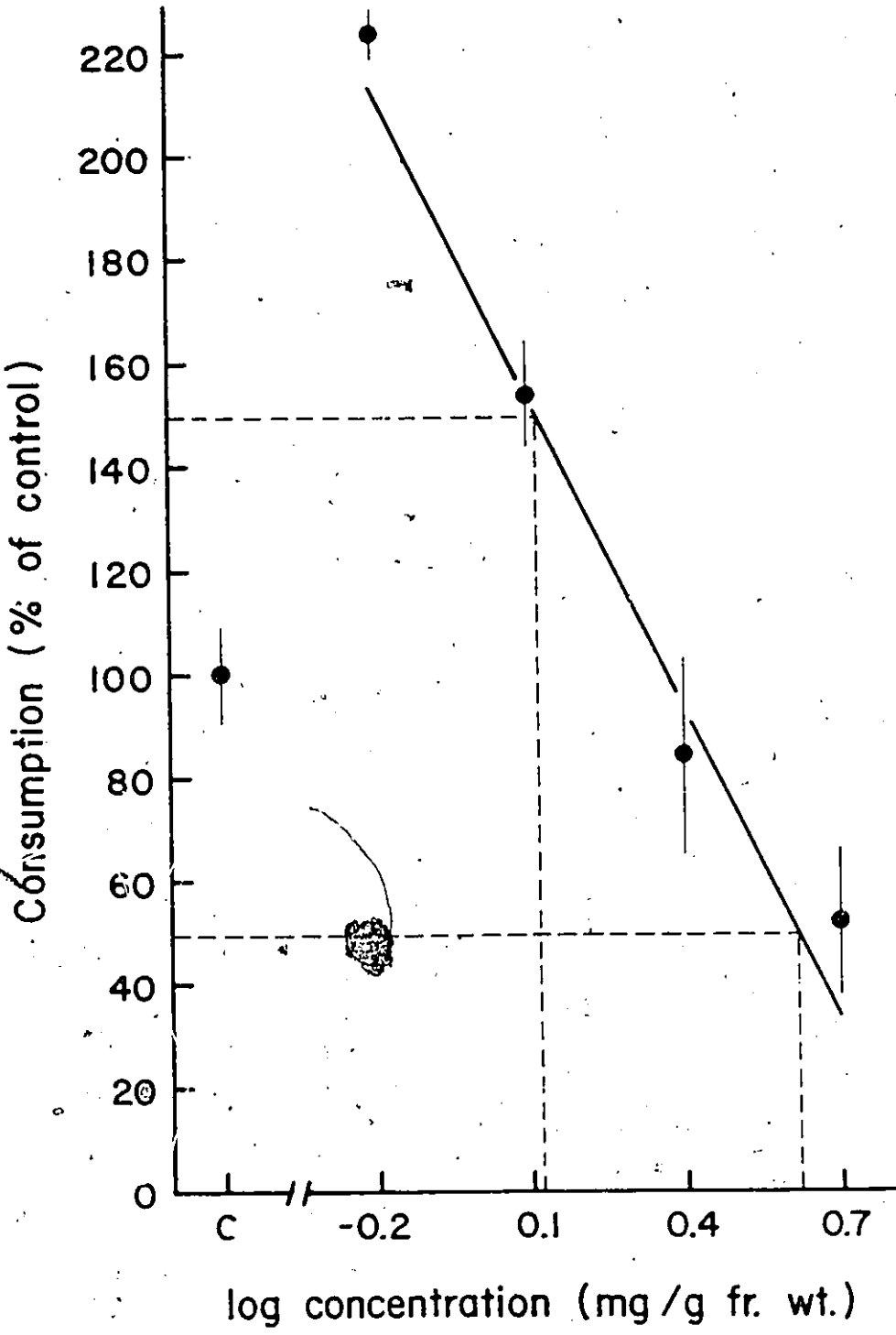
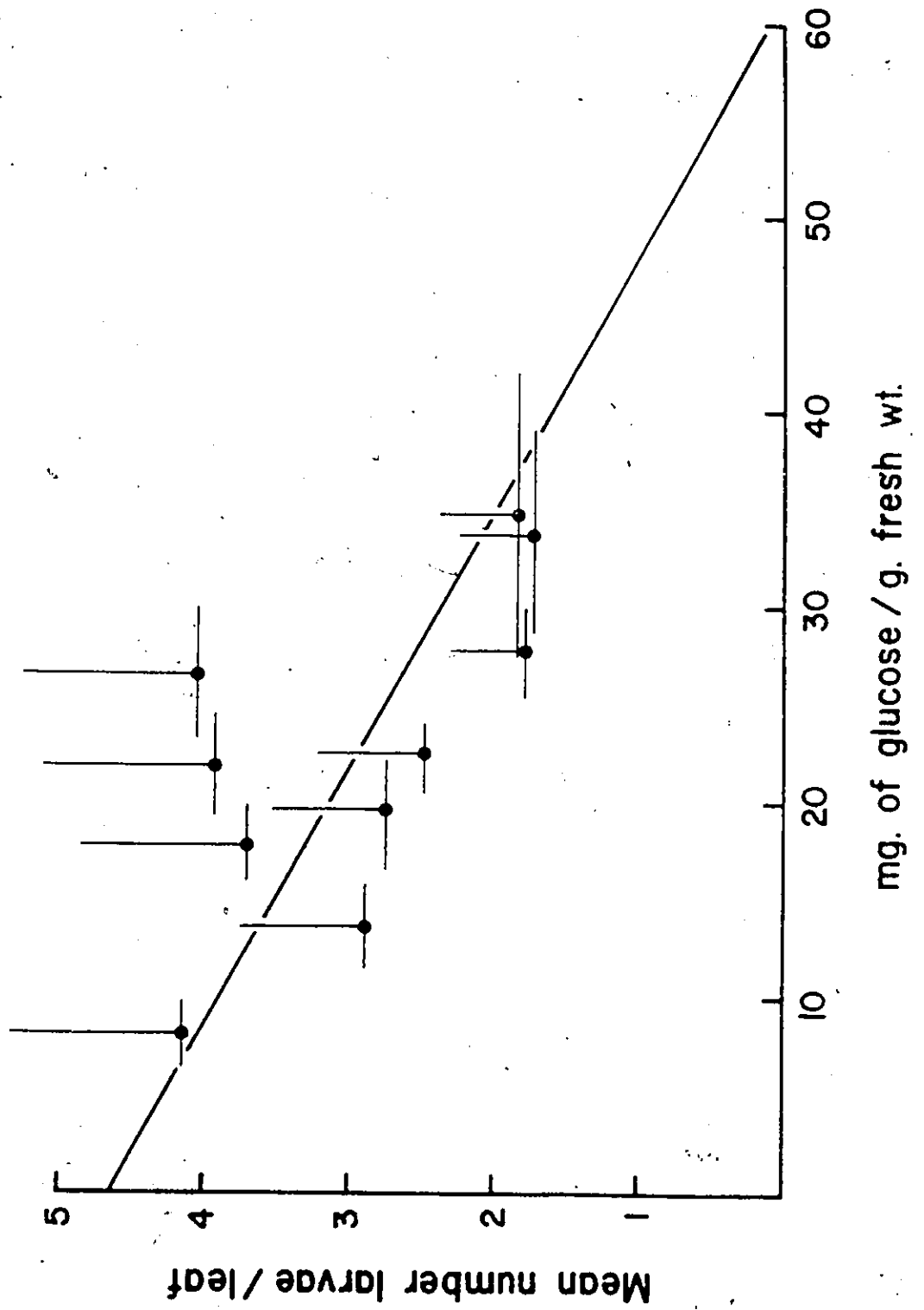


Figure 16: Relationship between infestation of tomato fruitmoth larvae (as sum of unparasitized and parasitized larvae in the field during 1984) per leaf and sugar content of tomato leaves at 98 days of age. Concentrations of sugar and mean total number of larvae/leaf were obtained from the cultivars under study. Error bars represent one standard deviation




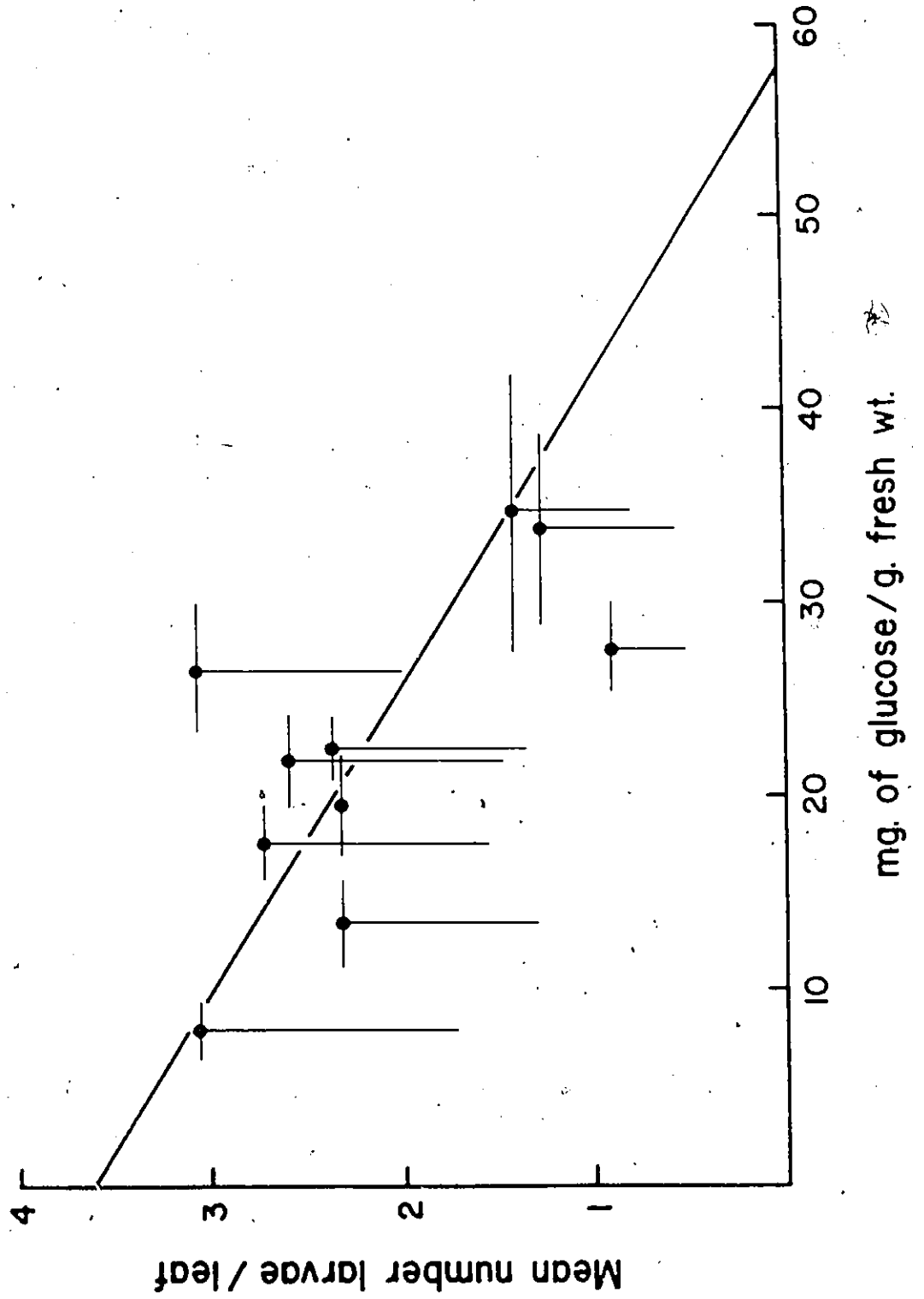


Figure 17: Relationship between infestation of tomato fruitmoth (as sum of unparasitized and parasitized larvae in the field during 1986) per leaf and sugar content of tomato leaves at 98 days of age. Concentrations of sugar and mean total number of larvae/leaf were obtained from the cultivars under study. Error bars represent one standard deviation



found with a marginal significance ($r = -0.55$, $p > 0.099$ for 1984 and $r = -0.46$, $p > 0.19$ for 1986). The lines fitted through a forward selection procedure for the variables under study are shown in Figure 18. In relation to consumption of leaf disks for each larval instar the correlation was low but always in an inverse relationship (Appendix 1C).

The correlation between the dependent variables in the study and the levels of rutin in leaves (Appendix 2C) shows a very low and not significant correlation (eg $p = 0.54$ in relation to consumption of leaf disks by third instar larvae and $p = 0.92$ in relation to the total larvae in the field during 1984) although it is important to note that a positive slope was found. Lines fitted through a forward regression procedure were determined (Figure 19 and 20) for consumption of leaf disks by *S. absoluta* in each larval instar and the level of rutin and chlorogenic acid in leaves. Although the significance levels were between $p > 0.15$ and > 0.74 (Appendix 1C and 2C) they were graphed to show the negative slope of chlorogenic acid and positive slope for rutin.

The catecholic phenolics had no significant correlation with the dependent variables. The r value for correlation with total number of larvae for 1984 was -0.41 with $p > 0.24$ and for 1986 $r = -0.37$ with $p > 0.28$. The r for the correlation between each larval instar and catecholic phenolics was between 0.05 and 0.32 , with $p > 0.53$ and 0.92 (Appendix 3C). As is shown in Appendix 4C, there were no significant correlations between total number of larvae in 1984 and 1986, and nitrogen content of leaves. The same results were obtained for consumption of leaf disks by each larval instar. When relationships among levels of catecholic phenolics, chlorogenic acid, rutin, sugar and nitrogen were examined, several significant correlations

Figure 18: Relationship between infestation of tomato fruitmoth (as sum of unparasitized and parasitized larvae in the field during 1984 and 1986) per leaf and chlorogenic acid content of tomato leaves at 98 days of age. Concentrations of chlorogenic acid and mean total number of larvae/leaf were obtained from the cultivars under study. Error bars represent one standard deviation

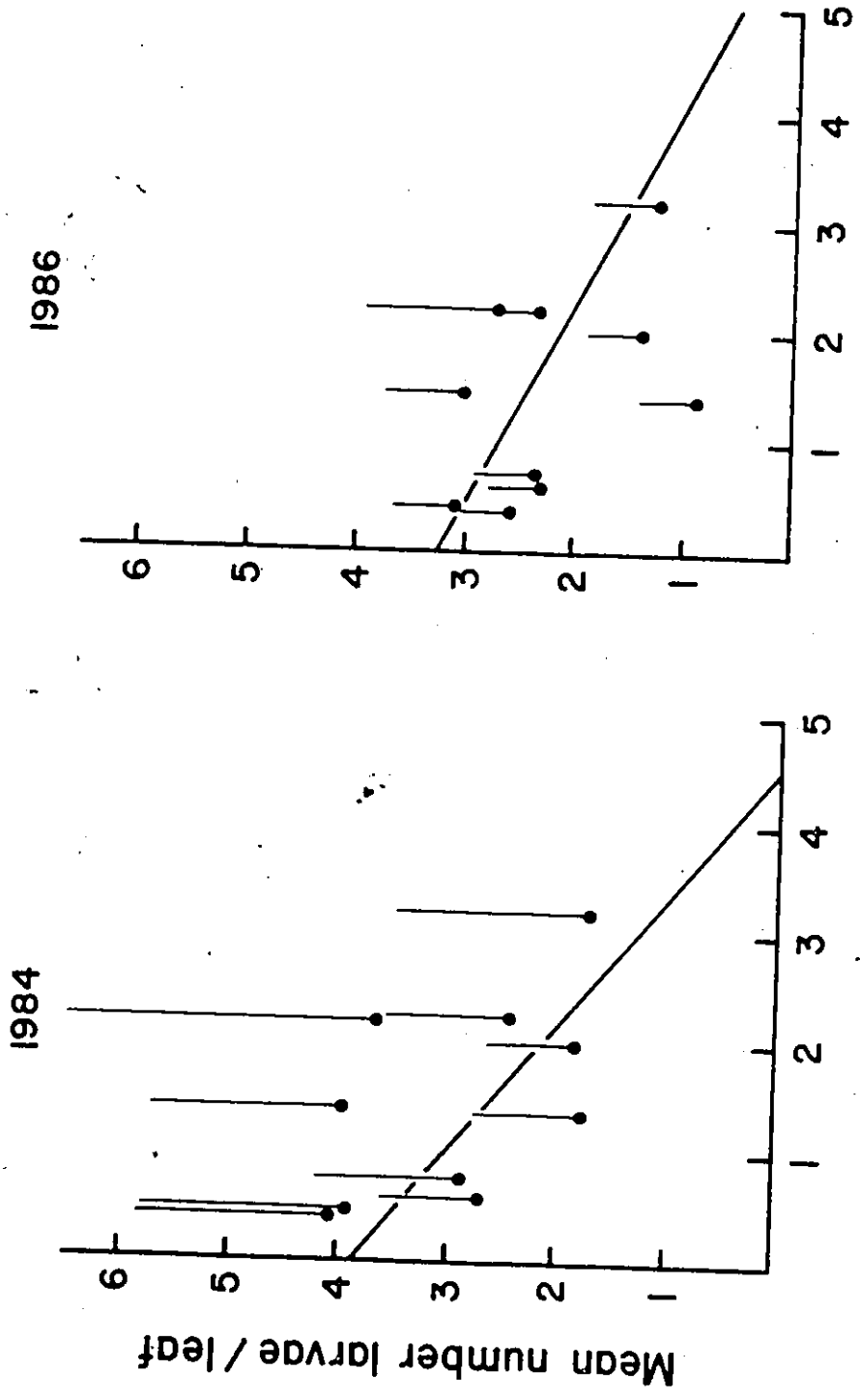


Figure 19: Relationship between consumption of tomato leaf disks by first and second larval instar of *S. absoluta* and chlorogenic acid and rutin content of tomato leaves at 98 days of age

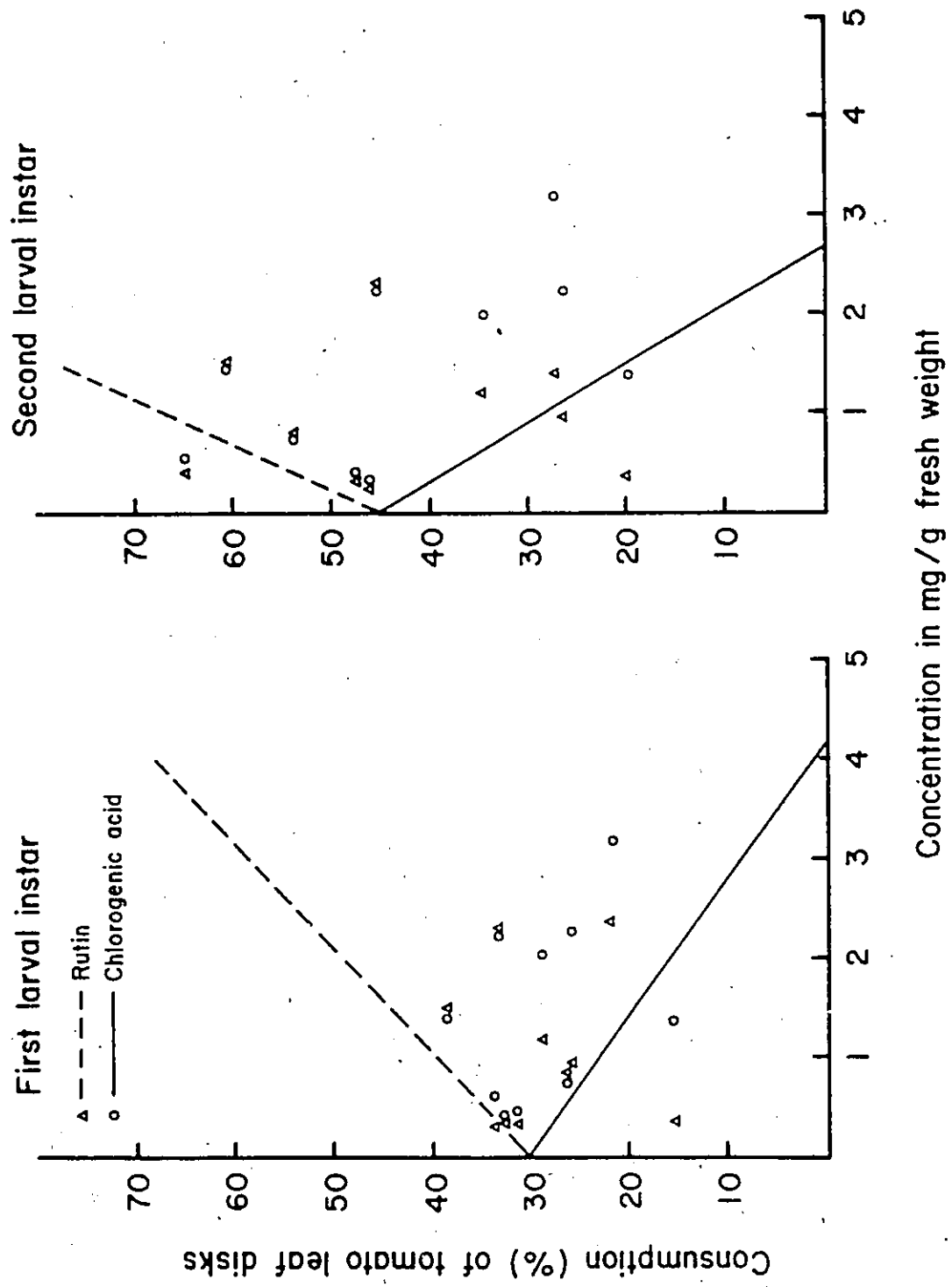
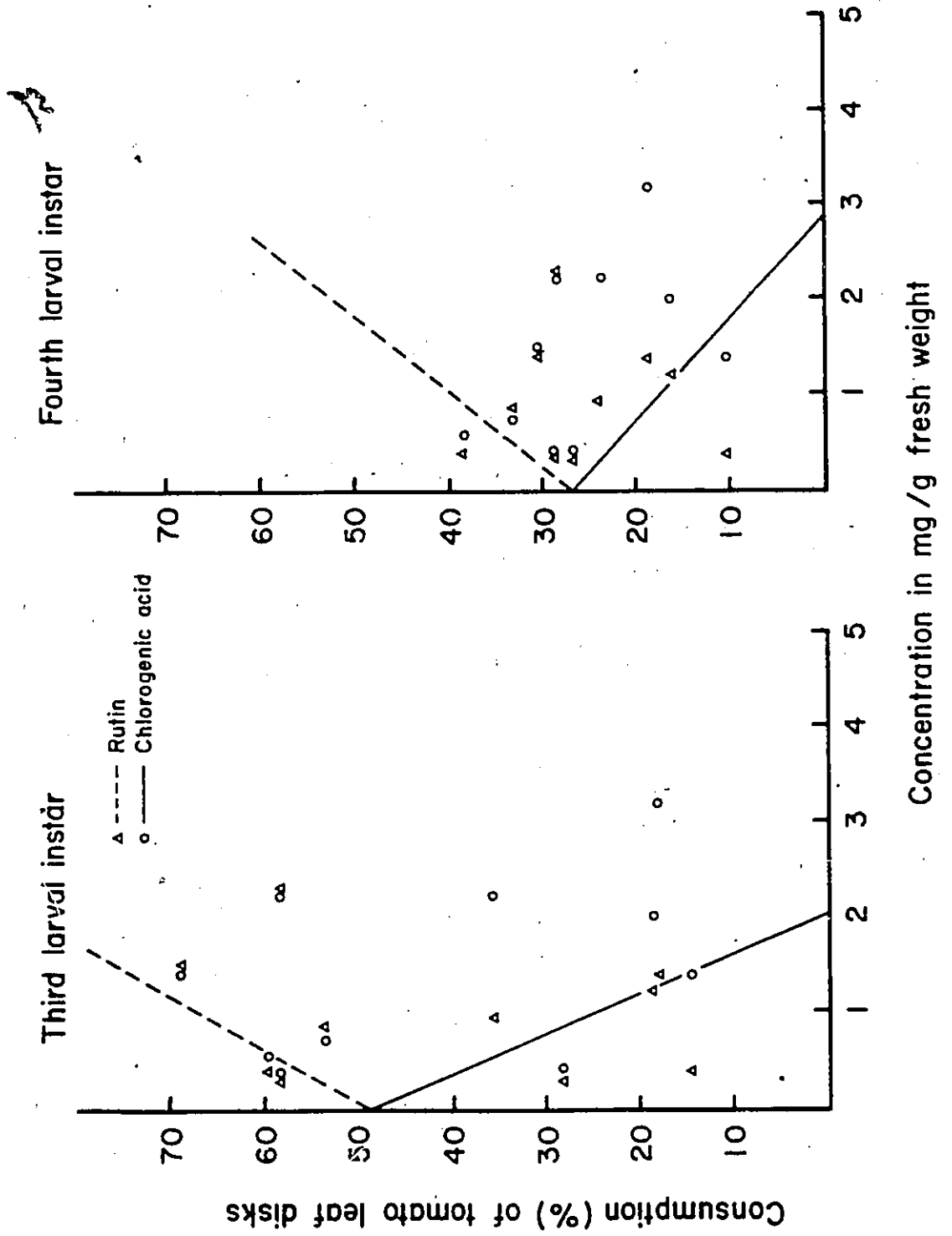


Figure 20: Relationship between consumption of tomato leaf disks by third and fourth larval of *S. absoluta* and chlorogenic acid and rutin content of tomato leaves at 98 days of age



were determined. Between levels of catecholic phenolics and chlorogenic acid a significant correlation was found with, $r = 0.65$ and $p = 0.04$. A significant correlation was also found between catecholic phenolics and rutin with $r = 0.65$ and $p = 0.04$. The total sugars analyses showed a significant correlation with catecholic phenolics with $r = 0.74$ and $p > 0.01$. Also, a correlation was determined between total sugar and chlorogenic acid ($r = 0.65$ $p > 0.039$). There was a significant correlation between the nitrogen content and the level of chlorogenic acid ($r = -0.65$ $p > 0.04$). However, significant correlations between nitrogen and other variable were not found.

3.10 Correlations Between Infestation by *S. absoluta* and Levels of Foliar Sugar

The negative correlation between susceptibility and sugar content was a striking result which required some reassessment of the experimental design. It is, of course, very unusual to find that a phagostimulant, as sugar, actually is correlated with decreased susceptibility. A possible explanation for this result is that the Dubois analysis for sugar was, in fact, detecting α tomatine, which is a glycoside containing two molecules of glucose and one each of galactose and xylose. To test this hypothesis a final experiment was undertaken to determine the effect of pure tomatine in the Dubois test. The Dubois test indicated that in the case of variety Ace 55 VF, which has been reported as having 0.76 mg tomatine/g fresh leaves (Elliger *et al*, 1981), there is yield of 0.537 mg/g fresh weight of glucose equivalents in the colorimetric reaction based on the tomatine contribution. This tomatine represents only 4% of glucose equivalents detected in extracts of this variety. If we consider that *Lycopersicon esculentum*, has been reported to contain between 60 and 400

mg/100 g fresh weight of α tomatine (Sinden *et al*, 1978), we could assume that in varieties with both high levels of sugars and the highest level of α tomatine (Table 14), only a 8% of the total sugar equivalents could be attributed to the presence of α tomatine. These results do not support the hypothesis that α tomatine is contributing significantly to the colour reaction in the Dubois test.

Chapter IV

Discussion

The present study has determined that within the commercial cultivars of *Lycopersicon esculentum* Mill. tested, there is a considerable and reproducible range of susceptibilities to attack by *S. absoluta* Meyrick larvae. Furthermore the development of natural enemies of this pest does not appear to be correlated to the tomato plant resistance. Thus, natural resistance and biocontrol can be combined in the pest management strategy. In an investigation of putative antifeedants and phagostimulants of *S. absoluta* in tomato cultivars, sugar a phagostimulant, is actually correlated strongly and significantly with decreasing susceptibility. Determination of total sugar by colorimetric methods might be an effective way to rapidly classify tomato plant varieties for differential resistance to *S. absoluta*.

4.1 Degrees of susceptibility of commercial tomato cultivars to *S. absoluta* (Meyrick)

In the present study degrees of susceptibility of tomato cultivars after infestation with *S. absoluta* larvae were classified on the basis of a multiple range analysis at a level of significance of $P=0.0001$. In the field, the cultivars that had the highest number of larvae per leaf were considered highly susceptible and the cultivars with the lowest significant number of total larvae per leaf were considered to be low in susceptibility. The cultivars that showed infestation levels between high and low susceptibility, with significant differences from these two groups, were considered to be of intermediate susceptibility.

Degrees of susceptibility and/or resistance are usually measured by the damage

that a specific insect causes under a given set of conditions (Horber, 1980; Mihm, 1983). However in the present study, a rating scale of damage in tomato leaves or fruits by *S. absoluta* larvae was not considered reliable. First, there was the problem of quantitatively assessing leaf area damaged by a microlepidopteran which produces small tunnels. Secondly, a high parasitization rate was observed (Table 5) in all the cultivars, which masked the total potential damage that tomato fruitmoth could have produced. For this reason, total larvae supported per leaf was the best measure of susceptibility.

The field test showed that cultivars Duke, Hybrid 6718 and land race Condino have low susceptibility to *S. absoluta* larvae, the cultivars Baron and Cal Ace have a high susceptibility. The other cultivars Laser, Hybrid 7718, Ace 55 VF, Pakmor and Petoeary were of intermediate susceptibility.

The degree of susceptibility in standardized laboratory tests was also determined on the basis of the multiple range analysis at a statistical level of $P=0.05$, using measurements of the amount of leaf area consumed by each larval instar of *S. absoluta* per cultivar. This test also showed significant differences among cultivars in relation to consumption of tomato leaf disks by *S. absoluta* larvae. The varieties found in the field to be less susceptible to tomato fruitmoth were also found with low susceptibility in laboratory feeding tests across all larval instars. However, the cultivar Baron found in the field as susceptible, showed an intermediate susceptibility, under laboratory conditions, while the other cultivars were found to be highly susceptible.

While no data is available about oviposition preference, artificial infestation in the field would tend to mask the natural infestation and reflect antibiosis more

than preference. Laboratory tests on consumption in a no choice situation are possibly also an indication of antibiosis (Renwick, 1983). Growth studies, which were not attempted because of the lack of a meredid diet and small size of larvae, may be a better index of antibiosis than consumption studies. On the other hand, analyses of correlations between infestation in the field for both years and feeding in laboratory conditions across all larval stages, showed a high level of significance ($r = 0.85$, $p > 0.002$ for 1984 and $r = 0.67$, $p > 0.033$ for 1986), which suggests similar mechanisms of resistance to *S. absoluta* operate under field and laboratory conditions. In addition, the result indicates that a rapid laboratory assessment of consumption is a predictable indicator of field performance.

The differences in tomato fruitmoth larvae populations in the field, or feeding behavior in the laboratory on different cultivars and the significant correlation found between these variables suggests the presence of some physical and/or chemical make up of tomato leaves that may be interacting with *S. absoluta*. The low number of larvae/leaf found in the land race Condino under field conditions can be explained in part by its physical characteristics: higher pubescence and tissue toughness compared to the other cultivars studied. However, physical differences among other cultivars was not established.

Condino is a land race obtained from "campesinos" from Rengo, Central Chile where it has been cultivated for a long time and is valued locally because this tomato land race has lower damage by *S. absoluta* than the commercial cultivars. The resistance is maintained by active selection by local farmers, who have observed its agronomic performance. However because of its moderate yields, Condino is not used commercially. The other resistant cultivars Duke and Hybrid 6718 both are

hybrids. Duke is known to be highly resistant to fungi, especially to *Verticillium* spp and *Fusarium oxysporum* var *lycopersici* race R1 and R2, widely distributed fungi in Chile. Hybrid 6718 is also known for its resistance to *Alternaria* stem canker (Petoseed, Chile, 1984). It is possible that the allelochemicals underlying plant resistance to pathogens in these varieties are also implicated in the resistance to *S. absoluta* larvae, as has been determined in other systems. For example, catechol is apparently a factor in disease resistance in onions, and has been found to be toxic to the greenbug, *Schizaphis graminum*, and to inhibit the growth and digestive physiology of the black cutworm (Bell, 1974). However, in the present study, Baron, a cultivar resistant to fungi, showed high susceptibility to *S. absoluta* in field. Therefore, resistance to fungi may not always be an indicator of resistance to insects.

The results of the present study show that plant resistance should be studied in relation to the biology of the insect pest, as well as the natural enemies that interact with the pest. The larval population remained low at the beginning of February and increased to a maximum in the beginning of March (plant age: 92 days). This result is to be expected considering that regular artificial infestation was employed. The higher maximum population in 1984 as compared to 1986 probably reflects higher natural infestation (in addition to the artificial infestation) in 1984 as compared 1986. In this study it was also clearly established that varieties with low susceptibility showed a more stable population of larvae than high susceptibility varieties. Another important feature of the population dynamics is that the increase in the larval population observed at beginning of March coincides with fruit set in late tomato production. The result is a high level of fruit damage, varying

from 60 to 100% if the cultivars are susceptible to *S. absoluta* (Matta and Ripa, 1981). This damage is compounded by the movement of larvae from foliage to fruits and new oviposition in fruits. The present observations of the dynamics of larval populations for the Aguila Sur locality (Paine, Metropolitan Region) are slightly displaced in relation to observations by Matta and Ripa, 1981 for the Quillota locality (V Region), where the maximum larval population was observed in mid February during the 1971 and 1972 season.

The time of planting of a particular variety and the dynamics of the fruitmoth population can influence the final damage observed. For example, Cal Ace, one of the cultivars found to be the most susceptible to *S. absoluta*, is also the most frequently utilized cultivar in later production in Central Chile. The combined increase of the tomato fruitmoth larval population during the beginning of March and the high susceptibility of Cal Ace explain why extremely high levels of damage occur often in this situation, Baron the other cultivar also found to be highly susceptible under field conditions, normally does not have serious problems with *S. absoluta* because it matures before the insect pest is at a maximum in Central Chile. However, it is very important to consider this susceptibility for Northern Chile where *S. absoluta* attacks tomato plants during the entire year (Vargas, 1970; Acuna, 1970). The less susceptible varieties such as Duke and Hybrid 6718 are used in early production in the field and greenhouse respectively, because of their biological characteristics in relation to pollen maturation and resistance to low temperatures. These are conditions where fruitmoth populations are low, and the consequent damage is very low because of combined resistance and low populations. Condino, the low susceptibility land race, is not produced commercially, but potentially could be used

late in the field, although yields may be generally lower than the cultivar Cal Ace. The threshold cost-benefit of this land race in comparison to other cultivars and *S. absoluta* control is beyond the scope of this study, but is an important consideration for future recommendations.

The effect that the introduction of resistant varieties can have on the third trophic level (parasitoids and predators), is an important factor to analyze in plant insect relationships. Natural enemies of a pest do not always possess ecological and physiological tolerances enabling them to survive physical or biochemical perturbations in the host plant (Philogène, 1982). Several studies (Campbell and Duffey, 1979; Thurston and Fox, 1972; Mc Dougall, 1986) have demonstrated the potential incompatibility of biological control with host-plant resistance programs that select for a high content of a chemical toxic to the pest. Campbell and Duffey, 1979 and 1980 showed that the naturally occurring glycoalkaloid in tomato, α tomatine, is toxic to an ichneumonid parasitoid of *Heliothis zea*. In the present investigation, larvae of *S. absoluta* were found parasitized in large proportion by the parasitoid *D. phthorimaeae* De Santis. A parasitization rate of 73.4% for 1984 was found and 19% in 1986. Rojas (1981) indicated that this parasitoid can parasitize up to 40% of *S. absoluta* in the field under natural conditions. Larrain (1986) suggested that *D. phthorimaeae* is the most effective natural enemy of *S. absoluta* in Central, Chile. The present investigation also determined that *D. phthorimaeae* was the most important parasitoid establishing on *S. absoluta* larvae, but its population is evidently highly variable from one season to the next, and in this study was higher than that found by Rojas, 1981. This could be explained by disturbances in the

phytophagous insect-natural enemy complex. The field test was undertaken in a region where the surrounding area under tomato cultivation was much higher in 1986 than 1984. Therefore, the intensive insecticide application during 1986 could have affected this parasitoid, because natural enemies are generally more susceptible to pesticides than their hosts or prey (Philogène, 1982). The results of the present study indicate that there is no correlation between the rate of *D. phthorimacae* parasitization of *S. absolutalarvae*, with susceptibility among varieties. This finding suggests that factor(s) conferring resistance in tomato cultivars to *S. absoluta* do not affect *D. phthorimacae*. Therefore, resistant varieties and biological control utilizing *D. phthorimacae* may be combined for the control of *S. absoluta* a finding that has considerable applied importance in an I.P.M. strategy.

4.2 Chlorogenic acid, rutin and total catecholic phenolic compounds in leaves of tomato cultivars and their relation with degrees of susceptibility to *S. absoluta* (Meyrick)

The resistance of tomato plants to insect pests has been shown in numerous investigations to be controlled by physical factors and/or phytochemicals (Cosenza and Green, 1979; Fery, 1974; Fery and Cuthbert, 1975; Barbosa, 1979; Schuster *et al* 1979, Elliger *et al*, 1981, Duffey and Isman, 1981; Isman and Duffey, 1982a,b). However, the present investigation suggested that only the land race Condino had important physical characters that could be involved in the resistance to *S. absoluta*

In the foliage of tomato plants and related wild and cultivated species, some chemical compounds have been demonstrated to confer resistance to some insect

pests (Fery and Cuthbert, 1975; Elliger *et al*, 1981; Duffey and Isman, 1981; Isman and Duffey, 1982a, 1982b). Phenolic compounds at concentrations present in tomato have been found to act as deterrents to *Heliothis zea* larvae (Lepidoptera: Noctuidae). In the present study, it was determined that chlorogenic acid at concentrations found in the cultivars under study (Table 9) (between 0.319 and 0.036% fresh weight) may contribute to but is not totally responsible for observations of *S. absoluta* under field and laboratory conditions. For example, the field results show an inverse relationship with a negative slope in both years between the number of larvae/leaf and level of chlorogenic acid in leaves. The r^2 value for 1984 was 0.30 and for 1986, 0.21, suggesting that 30% and 21% of the variation between cultivars in the two years can be explained by variation in chlorogenic acid. In addition, a direct measure of deterrent action of chlorogenic acid to *S. absoluta* larvae was observed in laboratory feeding assay where chlorogenic acid had an antifeedant effect on second instar larvae. The PC50 (the concentration for 50% inhibition of feeding) was 2.4 mg/g fresh weight, and this concentration is within the concentration range found in the cultivars under study. The land race Condino showed the highest level of chlorogenic acid with 3.19 mg/g fresh weight. The PC50 of chlorogenic acid to *S. absoluta* is comparable to *H. zea* (PC50=2.5 mg/g fresh weight) (Elliger *et al*, 1981; Isman and Duffey, 1982). Both the antifeedant activity of chlorogenic acid and correlations between field resistance and H.P.L.C. levels of chlorogenic acid in leaves suggest that the substance plays a role in resistance, but may not be the only factor involved.

The response of tomato fruitmoth to rutin was different from that observed with chlorogenic acid. The field test does not show any significant correlation between

number of larvae supported per leaf and levels of rutin in leaves, but it is important to note that its slope was positive. The laboratory feeding test showed that rutin, at observed intermediate levels in the tomato cultivars (0.32-0.6 mg/g fresh weight) could be phagostimulatory (EC50, the concentration for 50% stimulation of feeding, was 1.28 mg). None of the cultivars analyzed had rutin levels sufficiently high enough to act as phagodeterrents based on the antifeedant assay, where deterrence for 50% inhibition of feeding was observed at concentrations above 4.6 mg/g fresh wt. This result suggests that rutin functions as a kairomone, rather than an allomone, as has been observed with many allelochemicals in other systems (Beck and Reese, 1976; Bernays and Chapman, 1979). With rutin there are diverse responses with different insect species. For example, McFarlane and Distler (1982) and Neville and Luckey (1971) report that rutin enhanced larval growth of the cricket (*Acheta domestica*) when added to the diet of this insect. Similar responses to dietary concentrations of rutin were found in the boll weevil, *Anthonomus grandis* (Maxwell *et al.*, 1967).

Total catecholic phenolic compounds were neither related to levels of *S. absoluta* in the field, nor to feeding behavior under the laboratory conditions. Isman and Duffey (1982) reported that in five tomato cultivars analyzed, including Ace 55 VF, chlorogenic acid and rutin constituted about 60% of the total phenolic content. In the present investigation, in the land race Condino, chlorogenic acid and rutin constituted 64% of the catecholic phenolics compounds. The total catecholic analysis reveals the presence of other phenolics, for example, a caffeoyl derivative of aldaric acid and other flavonoid glycosides not identified, but reported to be present in tomato leaves (Elliger *et al.*, 1981; Duffey and Isman, 1981). However there was a

significant correlation between level of catecholic phenolics and content of chlorogenic acid and rutin, suggesting that catecholic content is strongly influenced by chlorogenic acid and rutin. For Ace 55 V.F., Isman and Duffey (1982) found a total phenolic content of 0.45% fresh weight using the same methodology as the present study. The value found in this study (0.44 %) is in close agreement with their finding. Further comparison confirms that cultivars analyzed in this study have levels of catecholic phenolics higher than those of the other cultivars studied by Isman and Duffey, and that these differences are not due to methodology. *H. zea*, the insect that these authors have studied, is present in Chile attacking corn, but its occurrence on tomato plant is only occasional. An explanation could be the high level of catecholic phenolic compounds found in the cultivars cultivated in Chile.

4.3 Relationship between sugar content of leaves of tomato cultivars and their susceptibility to *S. absoluta* (Meyrick)

The levels of sugar found in the tomato hybrid cultivars under study coincide with the range determined by Gent(1984), also for tomato hybrids (24.5 to 35.3 mg/g fresh wt) in greenhouse conditions, with the exception of the cultivar Baron, which showed the lowest level of sugar (8.1 mg/g fresh wt). On the other hand, his cultivar was the earliest producer. An explanation for the low sugar level is that fruit ripening produces a mobilization of sugar, resulting in decreases in sugar levels in leaves (Gent, 1984). Glucose and sucrose were the free sugars determined through paper chromatography, although Gent(1984) indicates fructose may also be commonly found in tomato.

The role of sugar and especially sucrose, as a general feeding stimulant to many

insect species is extremely well documented (Shoonhoven, 1982). However, in the present study, a significant correlation was not found between proportions of leaves consumed by *S. absoluta* larvae in laboratory conditions and level of sugar per cultivar, but an inverse correlation was found between the number of tomato fruitmoth larvae/tomato leaf under field conditions and sugar levels in tomato leaves. The cultivars with the highest levels of sugar in leaves showed a lower susceptibility to tomato fruitmoth larvae under field conditions, where some proportion of infestation by *S. absoluta* is natural. It is particularly striking that these compounds long recognized as phagostimulants, are correlated with low susceptibility in the infestation under field conditions. Any allelochemical in tomatoes inhibiting the growth of larvae and whose concentration is strongly and highly positively correlated with sugar levels could explain the observed results. Chlorogenic acid is one possibility, and a correlation between sugar content and chlorogenic acid exists, but the r^2 value, 0.42, although significant is not very high. Furthermore the correlation between field infestation and sugar content ($r^2 = 0.45$ in 1984 and 0.49 in 1986) is much higher than the correlations between field infestation and chlorogenic acid content ($r^2 = 0.30$, $r^2 = 0.21$ respectively), suggesting that some other factor than chlorogenic acid must also be involved.

A second candidate allelochemical which may be correlated with sugar levels and be responsible for the effect is α tomatine, a compound which has been found to inhibit feeding in Colorado potato beetle *Leptinotarsa decemlineata* (Sturchkow, 1961), and *Locusta* (Bernays and Chapman, 1979). α Tomatine is a glycoalkaloid whose content in tomato leaves has been reported as highly variable (range of 460 to 5100 mg/100 g dry-wt). The variation is apparently due to factors such as

plant genotype, its stage of development and seasonal condition (Sinden *et al*, 1978). Tomatine content is higher in most wild species than in commercial cultivars (Khun *et al*, 1950). Leaves of the tomato cultivars have α tomatine levels of 400-60 mg/100 g fresh wt. Elliger *et al* (1981) and Isman and Duffey (1982) showed that α tomatine in diet concentrations reduces the larval growth of *H. zea*, with ED50 between 0.40 and 0.90 mg/g fresh weight. In search of an alternate explanation to the phenomenon, α tomatine was tested for its effect on the colorimetric sugar test. This experiment revealed that α tomatine, which contains two molecules of glucose and one each of galactose and xylose (Roddick, 1974) only contributes between of 4 to 8% the concentration of sugar detected in Chilean tomato cultivars by the Dubois methodology.

At the commencement of the present study the analysis of α tomatine content in leaves was not considered as a possible factor of resistance to *S. absoluta* because previous investigations showed that α tomatine, in association with phytosterols in tomato plants, is inactivated as an insect toxin at both the level of herbivory and the third trophic level (Campbell and Duffey, 1979a; 1979b; 1981). Nevertheless, the result obtained in the present study suggest that the determination of α tomatine levels in tomato cultivars is warranted, and its interaction in the degree of susceptibility of tomato cultivars to *S. absoluta*, and, of course, with other inhibitors such as protease inhibitors (Ryan *et al*, 1986; Broadway *et al* 1986), should be considered.

On the other hand, the strong and significant correlation obtained between the susceptibility of tomato cultivars to *S. absoluta* and the sugar content of tomato leaves suggest one possible application of colorimetric methods as an effective way

to rapidly classify tomato plant varieties for differential resistance to *S. absoluta*.

4.4 Relationship between nitrogen content of leaves tomato cultivars and their susceptibility to *S. absoluta* (Meyrick)

Plant nitrogen is a component of plant tissue critical for phytophagous insects (Strong *et al*, 1984). Nitrogen is variously measured as total nitrogen, soluble nitrogen, amino acid levels or protein nitrogen, depending upon the author. In the present study the content of nitrogen in leaves (% N in g dry wt) was tested.

An increase of nitrogen content in plant often improves assimilation and growth efficiencies, rates of growth survival and reproduction of individual insects and populations, and the average population size increases (Onuf 1978; Prestidge, 1982). However, in the present investigation, no correlation was observed between the % nitrogen in the leaves and neither number of larvae/leaf in the field, nor the consumption of tomato leaf disks. Nitrogen had an inverse relationship with the level of chlorogenic acid in leaves. which is interesting to note, because this result suggests that high level of chlorogenic acid combined with low levels of nitrogen in tomato leaves could be negatively affecting the number of larvae supported in tomato cultivars. However much more experimentation is required.

In summary, the results of the present study suggest that the degree of susceptibility of tomato cultivars to *S. absoluta* can not at this time be clearly correlated with any one classical allelochemical. The hypothesis of a relationship between sugar and α tomatine in the tomato cultivars under study must be tested as the next step towards understanding the biological basis of the tomato plant-*S. absoluta* interaction.

Appendix 1A

Number total of *S. absoluta* (Meyrick) larvae
on all cultivars of tomato plants at different ages
in the field during 1984 and 1986

Mean of total larvae (total number of larvae/leaf) **

Plant age days 1984

Plant Age days 1986

92	5.17	a	92	2.81	a
85	4.10	b	85	2.68	a b
100	3.78	c	71	2.58	b
71	2.69	d	100	2.26	c
116	2.41	e	64	2.21	c
123	1.83	f	116	1.79	d
64	1.78	f	123	1.79	d
57	1.45	g	57	1.56	e

Mean 2.90

2.21

** Based on an assessment of all cultivars placed in a randomized split plot design with 4 replicates. Each figure represents the mean of 400 plants.

Means followed by the same letter in a column are not significantly different by Tukey's studentized-range test ($P = 0.0001$)

Appendix 2A

Infestation of tomato fruitmoth by parasitoid *Dineulophus phthorimaeae*
De Santis
on all cultivars of tomato plants at different ages in the field during
1984 and 1986

*Mean number of parasitized tomato fruitmoth larvae/leaf **

Plant age days 1984			Plant Age days 1986		
92	3.95	a	85	0.72	a
100	3.03	b	92	0.70	a
85	2.78	c	71	0.57	b
116	1.93	d	100	0.51	b
71	1.87	d	64	0.30	c
123	1.53	e	116	0.22	c d
64	1.08	f	57	0.19	d
57	0.87	g	123	0.14	d
	Mean	0.42		2.13	

** Based on an assessment of all cultivars place in a randomized split plot design with 4 replicates. Each figure represents the mean of 400 plants.

Means followed the same letter in a column are not significantly different in Tukey's studentized range test ($P = 0.0001$)

Appendix 3A

Correlation coefficient between parasitization rate of tomato fruitmoth larvae and susceptibility of varieties to tomato fruitmoth larvae (as total number of larvae) during 1984 and 1986

<i>Dependent variable</i>	<i>Independent variable</i>	<i>Correlation coefficient</i>
Parasitization rate 1984	Susceptibility 1984	$r = 0.23$ $p > 0.51$
Parasitization rate 1986	Susceptibility 1986	$r = 0.38$ $p > 0.39$

Appendix 4A

Infestation rate of *S. absoluta* (Meyrick) larvae on all cultivars of tomato plant at different ages, in the field during 1984 and 1986

*Mean number of unparasitized tomato fruitmoth larvae/leaf **

Plant age days 1984			Plant Age days 1986		
85	1.32	a	92	2.11	a
92	1.22	a	71	2.01	a b
71	0.82	b	85	1.95	a b
100	0.75	b c	64	1.90	b c
64	0.69	c d	100	1.76	c d
57	0.57	d e	123	1.64	d e
116	0.49	e	116	1.57	e
123	0.31	f	57	1.37	f
Mean	0.77			1.79	

* Based on an assessment of all cultivars place in a randomized split plot design with 4 replicates. Each figure represents the mean of 400 plants.

Means followed the same letter in a column are not significantly different in Tukey's studentized range test ($P = 0.0001$)

Appendix 1B

Consumption of leaf disk (%) by second larval instar of *S. absoluta* (Meyrick) on tomato leaves treated with different concentrations of chlorogenic acid

Concentration of chlorogenic acid mg/g wt	Consumption of leaf disks by second larval instar of <i>S. absoluta</i> as % of leaf disks *
0.0	42.4 a
0.8	35.3 a b
0.4	33.6 a b c
3.2	21.8 b c d
1.6	17.4 c d
6.4	11.4 d

* Each figure represents the consumption (%) on five leaf disks of five sibling second instar larvae per leaf with 4 replicates during 48 hours, under laboratory conditions.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

Appendix 2B

Consumption of leaf disk (%) by second larval instar of *S. absoluta* (Meyrick) on tomato leaves treated with different concentrations of rutin

Concentration of rutin mg/g wt	Consumption of leaf disks by second larval instar of <i>S. absoluta</i> as % of leaf disks *
0.6	51.7 a
1.2	35.5 b
0.0	23.1 c
2.4	19.5 c
4.8	10.9 d

* Each figure represents the consumption (%) on five leaf disks of five sibling second instar larvae per leaf with 4 replicates during 48 hours, under laboratory conditions.

Means followed by the same letter in a column are not significantly different by Duncan's multiple range test ($P = 0.05$)

Appendix 1C

Correlation coefficient between total number of larvae in the field and consumption of leaf disks (%) by four larval instars of *S. absoluta* in relation to content of chlorogenic acid in leaves at 98 days of age

<i>Dependent variables</i>	<i>Independent variable chlorogenic acid correlation coefficient= r</i>	
Total larvae 1984	-0.551	$p > 0.099$
Total larvae 1986	-0.456	$p > 0.185$
Consumption by first instar	-0.374	$p > 0.286$
Consumption by second instar	-0.367	$p > 0.296$
Consumption by third instar	-0.481	$p > 0.159$
Consumption by fourth instar	-0.383	$p > 0.273$

Appendix 2C

Correlation coefficient between total number of larvae in the field and consumption of leaf disks (%) by four larval instars of *S. absoluta* in relation to content of rutin in leaves at 98 days of age

<i>Dependent variables</i>	<i>Independent variable rutin correlation coefficient= r</i>	
Total larvae 1984	0.37	$p > 0.918$
Total larvae 1986	0.100	$p > 0.782$
Consumption by first instar	0.192	$p > 0.595$
Consumption by second instar	0.214	$p > 0.552$
Consumption by third instar	0.116	$p > 0.749$
Consumption by fourth instar	0.218	$p > 0.543$

Appendix 3C

Correlation coefficient between total number of larvae in the field and consumption of leaf disks (%) by four larval instars of *S. absoluta* in relation to content of catecholics phenolics in leaves at 98 days of age

<i>Dependent variables</i>	<i>Independent variable catecholics-phenolics correlation coefficient= r</i>	
Total larvae 1984	0.407	$p > 0.242$
Total larvae 1986	0.372	$p > 0.288$
Consumption by first instar	0.059	$p > 0.870$
Consumption by second instar	0.223	$p > 0.535$
Consumption by third instar	0.032	$p > 0.929$
Consumption by fourth instar	0.138	$p > 0.702$

Appendix 4C

Correlation coefficient between total number of larvae in the field and consumption of leaf disks (%) by four larval instars of *S. absoluta* in relation to content of nitrogen in leaves at 98 days of age

<i>Dependent variables</i>	<i>Independent variable nitrogen correlation coefficient= r</i>	
Total larvae 1984	0.206	$p > 0.567$
Total larvae 1986	0.172	$p > 0.634$
Consumption by first instar	0.190	$p > 0.599$
Consumption by second instar	0.195	$p > 0.590$
Consumption by third instar	0.214	$p > 0.553$
Consumption by fourth instar	0.230	$p > 0.523$

References

- [1] Acuña, J. 1970. Control químico de la polilla del tomate *Gnorimoschema absoluta* (Meyr.). *Idesia, U. del Norte (Chile)* 1:49-53.
- [2] Apablaza, J. 1984. Insect and mollusk pests incidence on seven vegetable crops grown in the Chilean V and metropolitan regions. *Ciencia e Investigación Agraria, U. Católica (Chile)* 11(1):27-34.
- [3] Bahamondes, L.A. and A.R. Mallea. 1969. Biología en Mendoza de *Scrobipalpula absoluta* (Povolny) (Lepidoptera: Gelechiidae) especie nueva para la República Argentina. *Rev. Fac. Cienc. Agrarias* 15:96-104.
- [4] Barbosa, S. 1974. Insecticide and host plant resistance studies with the tomato fruitworm, *Heliothis zea* (Boddie) on processing tomatoes with host plant resistance notes on the tomato pinworm *Keiferia lycopersicella* (Walsingham). *Annual Review of Entomology* 26:365.
- [5] Beck, S. and J.C. Reese. 1976. Insect plant interaction : Nutrition and metabolism. *Rec. Adv. Phytochem.* 10:41-92.
- [6] Beland, G.L. and J.H. Hatcher. 1976. Expression of antibiosis to the bollworm in two soybean genotypes. *J. Econ. Entomol.* 69:557-560.
- [7] Bell, E.A. 1974. Biochemical bases of resistance of plants to pathogens. In *Proceedings of the Summer Institute on Biological Control of Insects and Diseases*, F.G. Maxwell and F.A. Harris, Eds. Univ. Press, Jackson 453:462.
- [8] Bernays, E.A. and R.F. Chapman. 1978. Plant chemistry and acridoid feeding

- behaviour. In Biochemical Aspects of Plant and Animal Coevolution, pp.99-141. J.B. Harborne, Ed. Academic Press, London.
- [9] Bligh, E.G. and J.W. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Bioch. and Phys.* #8 Vol. 37:911-917.
- [10] Blout, B.K. 1933. The colouring matter on the stem of the tomato. *J. Chem. Soc.* 1933:1528-1529.
- [11] Broadway, R.M., S.S. Duffey, G. Pearce and C.A. Ryan. 1986. Plant proteinase inhibitors: A defense against herbivorous insects. *Ent. Exp. Appl.* 41: 33-38.
- [12] Buhr, H., R. Toball and K. Schreiber. 1958. Die Wirkung von einigen pflanzlichen Sonderstoffen insbesondere von Alkaloiden, auf die Entwicklung der Larven des Kartoffelkäfers (*Leptinotarsa decemlineata* Say). *Entomol. Exp. Appl.* 1:209-224
- [13] Campbell, B.C. and S.S. Duffey. 1979. Tomatine and parasitic wasps: Potential incompatibility of plant antibiosis with biological control. *Science* 205:700-702.
- [14] Campbell, B.C. and S.S. Duffey. 1981. Alleviation of a tomatine induced toxicity to the parasitoid, *Hyposoter exiguae*, by phytosterols in the diet of the host, *Heliothis zea*. *Journal of Chemical Ecology* 7(6):927-946.
- [15] Campos, L. and C. Klein. 1967. Ensayos de control de la polilla del tomate *Gnorimoschema absoluta* en la Chimba de Antofagasta. U. de Chile Fac. de Agronomía, Dep. Biología, Memoria Anual 1967:40-53.
- [16] Campos, R.G. 1976. Control químico del minador de hojas y tallos de la papa *Scrobipalpa absoluta* (Meyrick) en el Valle de Canete. *Rev. Peruana de Entomología* 19:102-106.

- [17] Carballo, R., C. Basso, I. Scatoni and F. Comotto. 1981. Ensayo para el control de *Scrobipalpa absoluta* (Meyrick) temporada 1980-1981. Rev. Tecn. Fac. Agron., Uruguay 50:41-46.
- [18] Cosenza, G. and H. B. Green. 1979. Behavior of the tomato fruitworm, *H. zea* (Boddie), on susceptible and resistant lines of processing tomatoes. Hort. Science 14(2):171-173.
- [19] Courtney, W. H. and V. N. Lambeth. 1977. Glycoalkaloid content of mature and green fruit of *Lycopersicon* species. Hort. Science 12: 550-551.
- [20] Chan, B. G., A. C. Waiss Jr., R. G. Binder and C. A. Elliger. 1978. Inhibition of lepidopterous larval growth by cotton constituents. Ent. Exp. Appl. 24:294-300.
- [21] Dahlman, D. L. and E. T. Hibbs. 1967. Responses of *Empoasca fabae* to tomatine, solanine and demissidine. Ann. Entomol. Soc. Am. 60:732-740.
- [22] Davies, A. M. C., V. K. Newby and R. L. M. Synge. 1978. Bound quinic acid as measure of coupling of leaf and sunflower-seeds proteins with chlorogenic acid and congeners loss of availability of lysine. J. Sci. Food Agric. 29:33-41.
- [23] Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28(3):350-356.
- [24] Duffey, S. S. and M. B. Isman. 1981. Inhibition of insect larval growth by phenolics in glandular trichomes of tomato leaves. Experientia 37: 574-576.
- [25] Elliger, C. A., B. G. Chan and A. C. Waiss Jr. 1980. Flavonoids as larval growth inhibitors. Structural factors governing toxicity. Naturwissenschaften 67:358-359.

- [26] Elliger, C.A., R.E. Lundin and W.F. Haddon. 1981. Caffeoyl esters of glucaric acid in *Lycopersicon esculentum* leaves. *Phytochemistry* 20(5) :1133-1134.
- [27] Elliger, C.A., Y. Wong, B.G. Chan and A.C. Waiss Jr. 1981. Growth inhibitors in tomato (*Lycopersicon*) to tomato fruitworm (*Heliothis zea*) *J. Chem. Ecol.* 7(4):753-758.
- [28] Fery, R.L. 1974. Resistance of tomato cultivars to the fruitworm (*Heliothis zea*). *Hort. Science* 9:469-470.
- [29] Fery, R.L. and F.P. Cuthbert, Jr. 1974. Resistance of tomato cultivars to the fruitworm *Heliothis zea* (Boddie). *Hort. Science* 9:469-470.
- [30] Fery, R.L. and F.P. Cuthbert, Jr. 1975. Antibiosis in *Lycopersicon* to the tomato fruitworm (*Heliothis zea*). *J. Am. Soc. Hortic. Sci.* 100:276-278.
- [31] Fontaine, T.D., G.W. Irving, Jr., R. Ma, J.B. Poole and S.P. Doolittle. 1948. Isolation and partial characterization of crystalline tomatine an antibiotic agent from the tomato plant. *Arch. Biochem.* 18:467-475.
- [32] Fraenkel, G.S. 1959. The raison d'être of secondary plant substances. *Science* 128:1466-1470.
- [33] Garcia, F. 1984. La lucha biológica y la cooperación entre países. Mesa Redonda Internacional de Control Biológico. Santiago, Chile 9-10 Julio 1984.
- [34] Gates Clarke, J.F. 1965. Microlepidoptera of Juan Fernandez Island. *Proc. U. S. Nat. Mus.* 17(3508):80-81.

- [35] Gent, M.P.N. 1984. Carbohydrate level and growth of tomato plants. *Plant Physiol.* 76:694-699.
- [36] Herrera, A.J.M. 1963. Problemas insectiles del cultivo de la papa en el Valle de Canete. *Rev. Peruana de Entomología* 6:1-9.
- [37] Hoover, J.D., S.H. Wender and E.C. Smith. 1977. Effect of phenolic compounds on glucose 6 phosphate dehydrogenase isoenzymes. *Phytochem.* 16: 199-200.
- [38] Horber, E. 1980. Types and classification of resistance. In "Breeding Plants resistant to insects" pp 15-21. F.G. Maxwell and P.R. Jennings, Eds. John Wiley and Sons
- [39] Irving, G.W. Jr., T.D. Fontaine and S.P. Doolittle. 1945. Lycopersicin, a fungistatic agent from the tomato plant. *Science* 102:9-11.
- [40] Isman, M.B. and S.S. Duffey. 1982a. Toxicity of tomato phenolic compounds to the fruitworm *Heliothis zea*. *Ent. Exp. Appl.* 31:370-376.
- [41] Isman, M.B. and S.S. Duffey. 1982b. Phenolic compounds in foliage of commercial tomato cultivars as growth inhibitors to the fruitworm, *Heliothis zea*. *J. Am. Soc. Hort. Sci.* 107(1):167-170.
- [42] Kato, M. 1978. Phenols as indispensable components of the synthetic diet of the silkworm, *Bombyx mori*. *Ent. Exp. Appl.* 24:485-490.
- [43] Kennedy, G.G. and W.R. Henderson. 1978. A laboratory assay for resistance to the tobacco hornworm in *Lycopersicon* and *Solanum* spp. *J. Amer. Soc. Hort. Sci.*

- [44] Kennedy, G.G., R.T. Yamamoto, M.B. Dimock, W.G. Williams and J. Bordner. 1981. Effect of day length and light intensity on 2-tridecanone levels and resistance in *Lycopersicon hirsutum* f *glabratum* to *Manduca sexta*. J. Chem. Ecol. 7(4):707-717.
- [45] Lange, W.H. and L. Bronson. 1981. Insect pests of tomatoes. Ann. Rev. Entomol. 26:345-371.
- [46] Larráin, P. 1986. Evaluación de la mortalidad total y parasitismo *Dineulophus phthorimaeae* (De Santis) (Hym. Eulophidae) en larvas de la polilla del tomate *Scrobipalpa absoluta* (Meyrick). Agricultura Técnica (Chile) 46(2):227-228
- [47] Levinson, H.Z. 1976. The defensive role of alkaloids in insects and plants. Experientia 32:408-411
- [48] Lourencao, A.L., H. Nagai and M.A. Teixeira Zullo. 1984. Fontes de resistencia a *Scrobipalpa absoluta* (Meyrick, 1917) em tomateiro. Bragantia Campinas 43(2):569-577
- [49] Matta, A. and Ripa, R. 1981. Avances en el control de la polilla del tomate *Scrobipalpa absoluta* (Meyr.) (Lepidoptera: Gelechiidae) I. Estudios de población. Agricultura Técnica (Chile) 41(2):73-77
- [50] Markam, R.K. 1982. Techniques of flavonoid identification. pp Biological Techniques series. Academic Press, (113pp) London.
- [51] Maxwell F.G., J.N. Jenkins and W.L. Parrot. 1967. Influence of constituents of the cotton plant on feeding, oviposition and development of the boll weevil. J. Econ. Ent. 60:1294-1297

- [52] Meyrick, E. 1917. Descriptions of South American Microlepidoptera. Trans. Ent. Soc. London, 44 pp .
- [53] McDougall, C.A. 1986. Response of *Diadegma terebrans* (Gravenhorst) (Hymenoptera: Ichneumonidae) to berberine and alphaterthienyl in the diet of its host, *Ostrinia nubilalis* (Hubner) (Lepidoptera Pyralidae) M. Sc. Thesis, University of Ottawa. 145 pg.
- [54] McFarlane, J.E. and M.H.W. Distler. 1982. The effect of rutin on growth, fecundity and food utilization in *Acheta domesticus* (L). J. Insect. Physiol. 28:85-88.
- [55] Mihm, J.A. 1983. Efficient mass-rearing and infestation techniques to screen for host plant resistance to fall armyworm *Sodoptera frugiperda*. Centro Internacional de Mejoramiento de Maiz y Trigo. Bull. Mexico. 16 pp.
- [56] Moraes de, G. and J.A. Normanha Filho. 1982. An outbreak of *Scrobipalpula absoluta* (Meyrick) in tomato crop in the semi-arid tropic. Pesq. Agrop. Bras., Brasilia 17(3): 503-504.
- [57] Moreira, J.O.T., F.M. Lara and M.G.C. Churata-Nasca. 1981. Occorencia de *Scrobipalpula absoluta* (Meyrick) (Lepidoptera-Gelechiidae) danificando tomate rasteiro em Jaboticabal-S.P. In: Congresso Brasileiro de Entomologia, 7, Fortaleza Resumos. 58 pp.
- [58] Neville P.F. and T.D. Luckey. 1971. Bioflavonoids as a new growth factor to the cricket, *Acheta domesticus*. J. Nutr. 101:1217-1224.
- [59] Onuf C.P. 1978. Nutritive value as a factor in plant-insect interactions with an emphasis on field studies. In: "The ecology of arboreal Folivores:." pp 85-96.

G.G.Montgomery, Ed. Smithsonian Institution Press, Washington D.C.

- [60] Pacora,R.J.F. 1982. Parasitismo observado sobre dos insectos plagas en el cultivo de la papa del Valle Chillan. Ann. Review of Applied Entomology 70:2208.
- [61] Pesez,M. and J.Bartos.1974. Colorimetric and fluorimetric analysis of compounds and drugs. Marcel Dekker Inc., New York, 672 pp.
- [62] Philogene,BJ.R. 198 . Successes and future prospects for host plant resistance in integrated control systems. In "The role of Biological Control in Pest Management". pp 42-61. G.Allen and A.Rada, Eds. University of Ottawa Press.
- [63] Pohronezny,K., V.H. Waddill, D.J. Schuster, R.M. Sonoda. 1986. Integrated Pest Management for Florida tomatoes. Plant Disease. 70(2): 96-102
- [64] Politis,J.1948. Distribution of chlorogenic acid in *Solanaceae* and the organs of these plants. Compt. Rend. 229:692-693.
- [65] Polvony,D.1975. On three Neotropical species of *Gnorimoschemini* (Lepidoptera:Gelechiidae) mining *Solanaceae*. Acta Universitatis Agriculturae 23:379-393.
- [66] Prada,M.A.R. and J.P.Gutierrez.1974. Contribucion preliminar al control microbiologico de *Scrobipalpula absoluta* (Meyrick) con *Neoplectana carpocapsae* Weiser y *Bacillus thuringiensis*. Berliner en tomate *Lycopersicon esculentum* Mill. Acta. Agron. 24:116-137.
- [67] Prestidge,R.A.1982. The influence of nitrogenous fertilizer on the grassland *Ouchenorrhyncha* (Homoptera).J.Appl. Ecol.19:735-749.

- [68] Quiroz, C. 1976. Nuevos antecedentes sobre la biología de la polilla del tomate *Scrobipalpula absoluta* (Meyrick) Agricultura Técnica (Chile) 36:82-86.
- [69] Quiroz, C. 1978. Utilización de trampas con hembras vírgenes de *Scrobipalpula absoluta* (Meyrick) (Lep. Gelechiidae) en estudios de dinámica de población. Agricultura Técnica (Chile). 38:94-97.
- [70] Razuri, V. and E. Vargas. 1975. Biología y comportamiento de *Scrobipalpula absoluta* Meyrick (Lep. Gelechiidae) en tomatera. Revista Peruana de Entomología. 18(1):84-89.
- [71] Renwick, J.A.A. 1983. Nonpreference mechanisms: Plant characteristics influencing insect behavior. In "Plant resistance to insects" pp 199-213. P.A. Hedin, Ed. A.C.S. Symposium series.
- [72] Ripa, R. 1981. Avances en el control de la polilla del tomate *Scrobipalpula absoluta* (Meyrick II). Ensayos de control químico. Agricultura Técnica (Chile) 41(3):113-119.
- [73] Roddick, J.G. 1974. The steroidal glycoalkaloid a tomatine. Phytochemistry 13:19-25.
- [74] Rojas, S. 1965. Identificaciones de insectos entomófagos. Agricultura Técnica (Chile) 25(1):39-40
- [75] Rojas, S. 1981. Control de la polilla del tomate: enemigos naturales y patógenos. Inv. y Progr. Agrop. La Platina INIA (Chile) 8:18-20.
- [76] Sanchez, R.H.A., G. Bravo Viana. 1969. Ciclo biológico del gusano minador

- de la papa *Scrobipalpula absoluta* (Meyrick) en el Departamento de Nariño, Revista de Ciencias Agrícolas (2):3-19.
- [77] Santis de, L. 1983. Un nuevo genero y dos nuevas especies de Eulofidos Neotropicales (Insecta, Hymenoptera). Revista Peruana de Entomología 26(1):1-4.
- [78] Sarmiento, J.M., V.R. Razuri. 1976. Control de *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae) en el cultivo de la papa. Rev. Peruana de Entomología 19:99-101.
- [79] Scagel R.F., R.J. Bandoni, J.R. Maze, G.E. Rouse, W.B. Schofield and J.R. Stein. 1984. Plants and evolutionary survey. 757 pp. Wadsworth Publishing Co. California.
- [80] Scardini, D.M.B., L.R. Ferreira and P.A. Galveas. 1983. Control of the tomato moth *Scrobipalpula absoluta* (Meyrick) in the state of Espirito Santo. Ann. Rev. Entomol. 71(5):3636
- [81] Schuster, D.J. 1977. Effect of tomato cultivars on tomato accessions to the tomato pinworm *Keiferia lycopersicella*. J. Econ. Entomol. 70:434-436.
- [82] Schuster, D.J., V.H. Waddill, J.J. Augustine and R.B. Volin. 1979. Field comparisons of *Lycopersicon* accessions for resistance to tomato pinworm and vegetable leafminer. J. Am. Soc. Hort. Sci. 104:170-172.
- [83] Schalk, J.M. and A.K. Stoner. 1976. A bioassay differentiates resistance to the Colorado potato beetle on tomatoes. J. Am. Soc. Hort. Sci. 101(1):74-76.
- [84] Schoonhoven, L.M. 1982. Biological aspects of antifeedants. Ent. Exp. Appl. 31:57-69.

- [85] Sinden, S.L., J.M. Schalk and A.K. Stoner. 1978. Effects of daylength and maturity of tomato plants on tomatine content and resistance to the colorado potato beetle. *J. Amer. Soc. Hortic. Sci.* 103(5):596-600.
- [86] Strong, D.R., J.H. Lawton and Sir Richard Southwood. 1984. *Insects on plants community patterns and mechanisms.* Harvard University Press. 313 pp
- [87] Sturckow, B. and I. Low. 1961. Die Wirkung einiger *Solanum*-alkaloidglykoside auf den Kartoffelkafer, *Leptinotarsa decemlineata* Say. *Ent. Exp. and Appl.* 4:133-142.
- [88] Symposium. 1984. *Hortalizas de exportación: Resultado de investigación y proyecciones.* Departamento de Ciencias Vegetales. Facultad de Agronomía Pontificia Universidad Católica de Chile. 328 pp.
- [89] Todd, G.W., A. Getahun and D.C. Cress. 1971. Resistance in barley to the greenbug, *Schizaphis graminum*. 1. Toxicity of phenolics and flavonoid compounds and related substances. *Ann. Ent. Soc. Amer.* 64:718-722.
- [90] Thurston, R. and P.M. Fox. 1972. Inhibition by nicotine of emergence of *Apanteles congregatus* from its host, the tobacco hornworm. *Ann. Entomol. Soc. Amer.* 65:547-550.
- [91] Vargas, H. 1967. Estudio de la biología y enemigos naturales de la polilla del tomate *Gnorimoschema absoluta* (Meyrick) en el Valle de Azapa. Universidad Católica de Valparaíso (Chile) (Tesis Ing. Agronomo). 85 pp.
- [92] Vargas, H. 1970. Observaciones sobre la biología y enemigos naturales de la polilla del tomate, *Gnorimoschema absoluta* (Meyrick) (Lep. Gelechiidae). *Idesia, U.*

- del Norte (Chile). 1:75-110.
- [93] Whittaker, R.H. and P.P. Feeny. 1971. Allelochemicals: Chemical interactions between species. *Science* 171:755-770.
- [94] Williams, W.G., G.G. Kennedy, R.T. Yamamoto, J.D. Thacker and J. Bordner. 1980. 2-tridecanone: A naturally occurring insecticide from the wild *Lycopersicon hirsutum* f. *glabratum*. *Science* 207:888-889.
- [95] Wolfenbarger, D.A. 1966. Variation in leafminer and flea beetle injury in tomato varieties. *J. Econ. Entomol.* 59:65-68.