EVALUATION OF PHYTOALEXINS AND OTHER PLANT NATURAL PRODUCTS AS PROTECTIVE FUNGICIDES AND INSECT REPELLENTS

by

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Evaluation of Phytoalexins and Other Plant Natural Products as Protective Fungicides and Insect Repellents

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ABSTRACT

The potential use of phytoalexins as fungicides was evaluated against four foliar pathogens. All tests were carried out using a crude extract containing a mixture of phytoalexins from seeds of Phaseolus vulgaris. Complete protection resulted when the extract was added to fungal inocula. Preinoculation treatments with the extract at intervals of 2 h to 10 days gave 95 to 75 % protection but all inoculated plants eventually succumbed to infections which developed on epicotyl tissue. Postinoculation treatments gave no evidence for eradicative activity of the extract once fungal penetration had occurred.

Various plant extracts were evaluated as repellents and/or deterrents to Hylemya antiqua. Bean seed extracts containing phytoalexins deterred oviposition of H. antiqua in response to onion volatiles in a dual-choice bioassay. Bean seed extracts without phytoalexins (BSE) were equally or more deterrent. Deterrent activity of BSE persisted at least 4 days in dual-choice tests. Activity was affected by concentration; BSE was stimulatory to H. antiqua at 10^4 dilution. Oviposition deterrent activity was subsequently found in extracts of other tissues of P. vulgaris, as well as soybean seeds, tomato plants, grass leaves and onion and garlic

bulbs. Application of onion extract to onion bulbs markedly reduced oviposition. Onion seedlings growing in soil in the laboratory were protected from maggot damage by a spray treatment with a bean seedling extract. Results are discussed with respect to current host-selection theories and potential practises for pest control.

dedicated to the memory of

dear Mom whose long-term

sacrifices made possible

my educational

pursuits

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Finally, I am indebted to Jesus Christ, Son of God, Saviour and Lord, who gave His Life for me and who has been the Source of my personal strength in overcoming many obstacles.

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INTRODUCTION TO THE THESIS

Phytoalexins are antifungal compounds produced by plants in response to disease or nonspecific injury. Their induction, antifungal activity, implicated involvement in mechanisms of disease resistance, and metabolism by various microorganisms has been extensively studied and reviewed (19, 27, 39, 60, 66, 79, 80, 82, 134, 145). In contrast, research on the potential use of phytoalexins as exogenously applied chemicals for control of plant diseases or other pests has been limited (151), perhaps due to past difficulties in obtaining large amounts of phytoalexins. More recently, techniques which yield large quantities of phytoalexins from rotting plant tissue have been developed (74, 75, 151). As a consequence, I undertook to evaluate phytoalexins from Phaseolus vulgaris L. for potential use as fungicides.

A second objective of my research was to evaluate the effects of phytoalexins from P. vulgaris on insects. This latter objective was prompted by some similarities in structure and properties between pterocarpan phytoalexins (e.g. phaseollin) and rotenoids, both of which are natural products of plants in the Family Leguminosae, and by the fact that a casual preliminary test of phytoalexins for insecticidal activity (107) revealed apparent repellent activity against milkweed bugs.

CHAPTER 1

EVALUATION OF PHYTOALEXINS FROM *PHASEOLUS VULGARIS* L. AS PROTECTIVE FUNGICIDES AGAINST SELECTED FOLIAR PATHOGENS

INTRODUCTION

Antifungal activity of phytoalexin compounds has been demonstrated via bioassays involving spore germination or germ tube growth (13, 32, 58, 133, 142), radial mycelial growth on agar (12, 13, 26, 132, 142), mycelial dry weight in liquid culture (54, 56, 126, 127), and fungal growth on developed thin-layer chromatograms (10, 11, 67, 129). Correlative evidence suggests that phytoalexins function as antifungal agents in plants since they accumulate in host cells concurrent with the restriction of invading fungal hyphae in disease interactions (3, 10, 13, 18, 29, 31, 34, 52, 59, 72, 73, 78, 81, 83, 87, 100, 104, 106, 108, 113, 116, 125, 130, 139, 152).

Additional properties of phytoalexins which favour their potential development as fungicides include (151):

1. variability of molecular structure which offers scope for selectivity,
2. widespread occurrence,
3. biodegradability, and
4. potential for increased activity through chemical modification in the laboratory.

Ward and associates (151) were the first to report disease control with the application of phytoalexins as fungicides. They tested the protective activity of the phytoalexin capsidiol (from peppers) on the tomato - Phytophthora infestans (Mont.) Dby. host-parasite complex, and estimated the dilution

endpoint for complete protection at ca. 1.0×10^{-3}M capsidiol. Ward and associates (151) reported $\geq$ 80 % protection of treated plants for treatment-inoculation intervals ranging from 0 to 8 d (days). These results encouraged me to conduct a similar evaluation of phytoalexins produced by the garden bean, P. vulgaris.

Of the numerous antifungal compounds originating in P. vulgaris, those which have been isolated and identified are phaseollin (30, 101), phaseollinisoflavan (11, 23), kievitone (11, 131, 132) and phaseollidin (102). All four compounds are extractable with organic solvents from necrotic bean tissue. Three of the compounds have UV absorption maxima in ethanol at ca. 280 nm; kievitone has a broad absorption band with a maximum at 293 nm (11).

My evaluation of bean phytoalexins as potential fungicides included the following objectives:

1. indirect determination of fungitoxicity of PAE (phytoalexin extract) added directly to inocula (spore suspensions of common vegetable pathogens) via effects on disease symptoms,
2. determination of potential protective fungicidal activity of PAE (with and without adjuvant) for various preinoculation-treatment time intervals, and
3. determination of potential eradicative activity of PAE applied at various times after inoculation of plants.

MATERIALS AND METHODS

A. Phytoalexin Extract (PAE)

To prepare PAE, 1.2 kg of dry seeds of P. vulgaris 'Topcrop' were soaked in tap water for ca. 20 h at room temperature (RT= 22 to 24 C). Seed coats were then removed, and the hydrated seeds (2.4 kg) were chopped into pieces $\leq$ 5 mm in diam. The pieces were rinsed in tap water until the washings were clear. Cladosporium cucumerinum Ell. and Arth. was used to elicit phytoalexin accumulation in seed pieces. A dense spore suspension was prepared from 2-wk-old cultures of the fungus grown on potato dextrose agar by adding distilled water to each 9-cm petri dish culture and rubbing the culture surface with a glass rod. Spore suspension collected from 4 dishes was mixed with the seed pieces, and then drained. The inoculated seed pieces were spread in 2-cm deep layers on filter paper contained in covered glass dishes 20 cm diam x 8 cm deep, and kept in the dark for 4 d at RT. A 50-ml beaker filled with distilled water in each dish provided humidity.

After 4 d the seed pieces, now dark rusty brown in colour, were extracted 3 times with acetone at RT (1.5 liters for 12 to 24 h each time). The acetone extracts were filtered, combined, and concentrated under vacuum at less than 40 C to a syrupy residue (ca. 400 ml). The residue was extracted 3 times with equal volumes of ethyl acetate. The ethyl acetate fractions

were combined and taken to dryness under vacuum at less than 40 C. The residue was dissolved quantitatively in 200 ml of 95 % ethanol to give the PAE test solution.

Phytoalexins in this extract were quantitatively estimated by the thin-layer chromatographic method described by Rahe (1973). The main phytoalexins in PAE were phaseollin, phaseollinisoflavan and kievitone at ca. 3.0, 3.3 and 5.6 µg/µl, respectively. The amounts of the latter two compounds are not absolute, but based on absorbance values relative to that of a purified phaseollin standard.

Unless specified otherwise, PAE was applied to plant surfaces with the aid of an atomizer as a suspension in water at the indicated concentrations.

B. Growth of Plants

Seeds of P. vulgaris 'Topcrop' were planted in plastic pots containing vermiculite moistened to field capacity with Arnon Hoagland nutrient solution and placed in the dark. When seedlings had emerged, the pots were placed on a laboratory bench at RT under fluorescent lights (14:10 LD). Eight- to 20-d-old bean plants at equivalent heights and with fully expanded primary leaves were selected for treatment and for inoculation.

Corn (Zea mays L. 'Idahybrid 300'), tomato (Lycopersicon esculentum Mill. 'Bonny Best'), and cucumber (Cucumis sativus L. 'Straight Eight') were grown from seed in plastic pots filled with a soil:perlite mixture. Fertilizer (20:20:20, Green Valley Fertilizer and Chemical Co. Ltd., Surrey, B.C.) was included in the watering regime at the specified rate every second week. The plants were maintained in a greenhouse at conditions which ranged from 22 to 25 C with 13 h fluorescent-supplemented daylength in the winter to 25 to 35 C with natural daylength during the summer. For preliminary experiments, corn plants were 30 to 60 cm high at the time of treatment and inoculation; in later experiments corn plants were utilized when 15 to 30 cm high. Tomato and cucumber plants were treated and inoculated when plants possessed 4 to 6 fully expanded leaves.

C. Fungal Cultures and Inoculation

Colletotrichum lindemuthianum (Sacc. and Magn.) Scribner race beta and Colletotrichum lagenarium (Pass.) Ell. and Halst. race 1 were grown on bean juice agar (9); Cladosporium fulvum (Cooke) and Helminthosporium carbonum Ullstrup were grown on potato dextrose agar. Cultures were maintained at RT. Spores were harvested when cultures were 9 to 14 d old (C. lindemuthianum) or 3 to 4 wks old (H. carbonum, C. fulvum and C. lagenarium) by pouring about 10 ml of water onto culture

surfaces, rubbing gently with a glass rod to free spores, and filtering the resulting suspensions through 6 to 8 layers of cheesecloth. Spore concentrations were estimated with a haemocytometer and adjusted to ca. 1.0×10^6 conidia/ml for C. lindemuthianum and C. lagenarium, 4.0×10^5 conidia/ml for C. fulvum and 1.0×10^5 conidia/ml for H. carbonum. Inoculum was sprayed evenly as a fine mist onto bean stems or tomato, cucumber or corn leaves. Inoculated plants were first kept in humid chambers at RT and ca. 100 % RH for 48 to 65 h (bean), 45 h (tomato and cucumber), or 15 to 22 h (corn). Humidity chambers were opened for 10 to 24 h to allow equilibration of chamber and external atmospheres and drying of plant surfaces. Plants were then removed and placed under growth lights on a laboratory bench. Symptoms appeared within 36 h after inoculation of corn with H. carbonum, within 7 d after inoculation of bean and cucumber with C. lindemuthianum and C. lagenarium respectively, and within 14 d after inoculation of tomato with C. fulvum.

D. Phytoalexin Extract (PAE) Treatments

1. 'Inoculum plus Fungitoxicant' Bioassay. Initial tests for protective fungicidal activity of phytoalexins involved adding PAE directly to aqueous spore suspensions at a final concentration of 5 % (v/v). Inoculated control plants were sprayed with spore suspensions amended with 5 % of either

95 % ethanol or water. Uninoculated controls were sprayed with either 5 % PAE (v/v) or 5 % ethanol (v/v) in water at the time of inoculation.

2. Test for Eradicant Activity. PAE (5 %) or 5 % ethanol was applied to bean hypocotyls at intervals (1, 6, 31, 55, 79h) after inoculation with C. lindemuthianum. Epidermal strips from hypocotyls of untreated plants were examined microscopically at equivalent intervals after inoculation to follow the development of the infecting fungus with time.

3. Tests for Protectant Activity. The persistence of protection afforded by PAE applied to P. vulgaris or Z. mays at various intervals prior to inoculation with their respective pathogens was evaluated on the basis of qualitative criteria and quantitative estimates of symptoms. Control of the various factors contributing to the variability of host response in the P. vulgaris - C. lindemuthianum interaction was attempted by the use of 3 different methods to produce PAE-treatment-inoculation intervals.

(a) Method I. A large number of P. vulgaris seeds was planted, 5 % PAE (treatment) or 5 % ethanol (control) was applied to all plants 8 or 10 d later, and sets of treated and control plants were inoculated at various intervals after application of PAE.

(b) Method II. Planting of P. vulgaris was staggered, PAE or ethanol was applied when each plant was 10 d old, and all plants were inoculated when the oldest plant was 20 d old.

(c) Method III. PAE or ethanol was applied 8 to 14 d after planting of P. vulgaris and all plants were inoculated with a single spore suspension on the 14th day after planting. The effect of adjuvant on the protection afforded by both PAE (5 %) and Captan® (1 ppm; N-trichloromethylthio-tetrahydrophthalimide, Chevron Chemical Canada, Ltd.) on P. vulgaris or Z. mays was evaluated using Method III. Later's Spreader-Sticker (Later's Chemicals Ltd., Richmond, B.C.) was added to preinoculation spray treatments at 3 different concentrations (1500, 2500, 3500 ppm). Some inoculated control plants were sprayed with ethanol plus spreader-sticker at preinoculation intervals; others received no pretreatment.

E. Collection and Treatment of Data

Numbers of lesions which developed on bean hypocotyls as a result of compatible interactions between P. vulgaris and C. lindemuthianum were recorded 4.5 to 6 d after inoculation. The upper limit for counting of necrotic sites on hypocotyls of beans was arbitrarily fixed at 300 sites; larger numbers were recorded as 300+. Percent protection for treated

plants was calculated according to the formula:

$$\left[1 - \left(\frac{Lt}{Lc}\right)\right] \times 100 = \% \text{ protection}$$

where Lt = average number of lesions for treated plants

Lc = average number of lesions for control plants

Where rate of disease development was measured, percent of control for both treatment and control plants was calculated according to the formula:

$$\frac{Lx}{Lc} \times 100 = \% \text{ of control}$$

where Lx = average number of lesions at each observation time for treated or for control plants

Lc = average number of lesions at final observation time for control plants

Symptoms of the corn - H. carbonum disease interaction were quantitatively recorded 12 d after inoculation. Percent leaf or plant mortality for treated and control corn plants was calculated according to the formula:

$$\frac{\text{Number of dead leaves (or plants)}}{\text{Total number of leaves (or plants)}} \times 100 = \% \text{ leaf (or plant) mortality}$$

Qualitative comparisons of treated and control cucumber and tomato plants were recorded photographically 9 to 14 d after inoculation.

RESULTS

A. Inoculum plus Fungitoxicant (PAE)

Bean, corn, cucumber and tomato plants inoculated with C. lindemuthianum, H. carbonum, C. lagenarium, and C. fulvum, respectively were all protected by 5 % PAE (Figs. 1.1 to 1.3). When PAE was added to inocula at different levels, dilution endpoints were estimated to be 0.1 to 1.0 % PAE (12 to 120 ppm identified phytoalexins content) for complete protection against C. lindemuthianum on beans, and 1.0 to 2.5 % against H. carbonum on corn. Dilution endpoints for complete protection by Captan® tested in a similar manner against C. lindemuthianum and H. carbonum on their respective plant hosts were estimated to be 0.001 to 0.01 ppm and 0.01 to 0.1 ppm, respectively. Microscopic observation of epidermal strips from bean hypocotyls inoculated with C. lindemuthianum showed that conidia from inoculum containing 2.5 % PAE had not germinated and their walls appeared damaged.

B. Eradicant Activity

Protection was noted on plants treated with PAE at 1 and 6 h after inoculation; treatment was significant ($P < 0.01$) only for the 1 h interval (Table 1.1). The stages of development of C. lindemuthianum on hypocotyls of untreated plants at the various times of PAE-treatment are shown in Table 1.2.

Fig. 1.1 Protection of Phaseolus vulgaris by PA(=PAE) contained in inocula of Colletotrichum lindemuthianum at levels of 5 % and 1 %.

Control = uninoculated plants

H_2O Control = inoculum amended with 5 % water

Etoh Control = inoculum amended with 5 % ethanol

5 % PA = inoculum amended with 5 % PAE

1 % PA = inoculum amended with 1 % PAE

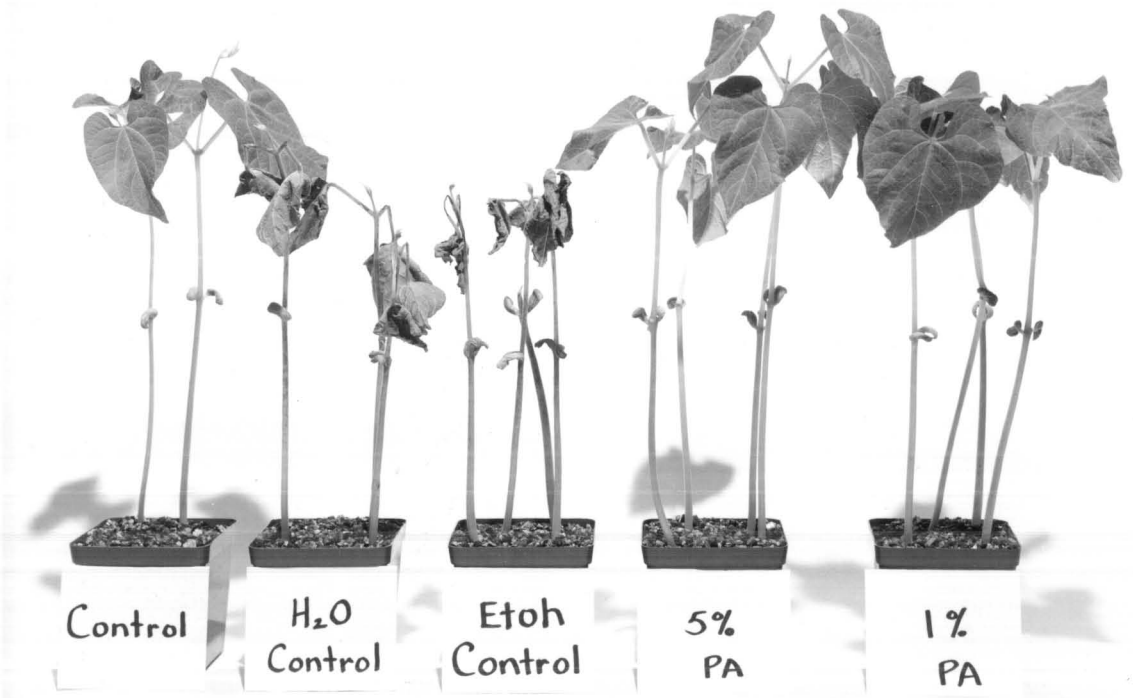

Control
H_2O Control
Etoh Control
5% PA
1% PA

Fig. 1.2 Protection of *Zea mays* by 5 % PA(=PAE) contained in inocula of *Helminthosporium carbonum*.

H_2O Control = uninoculated plants

H. carbonum H_2O control = inoculum amended with 5 % water

H. carbonum Etoh control = inoculum amended with 5 % ethanol

H. carbonum 5 % PA = inoculum amended with 5 % PAE

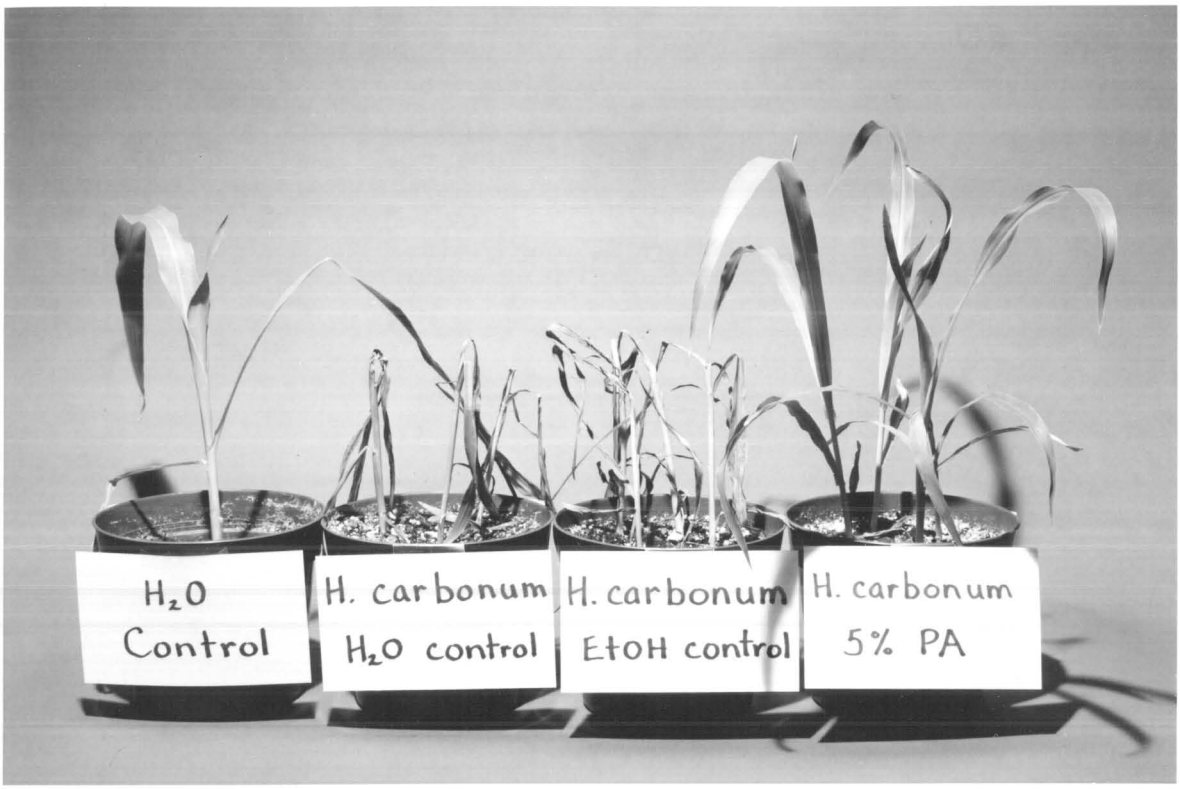
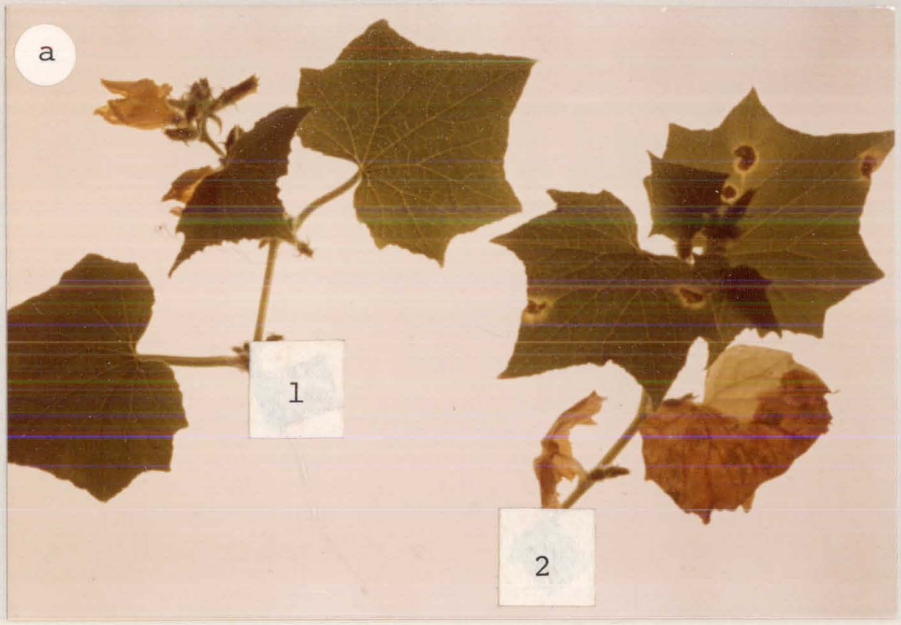

H_2O Control
H. carbonum H_2O control
H. carbonum EtOH control
H. carbonum 5% PA

Fig. 1.3 Protection of (a) Cucumis sativus by 5 % PA(=PAE) contained in inocula of Colletotrichum lagenarium and (b) Lycopersicon esculentum by 5 % PA(=PAE) contained in inocula of Cladosporium fulvum.

1. Treatment = inoculum amended with 5 % PAE
2. Control = inoculum amended with 5 % ethanol


a
1
2


b
1
2

TABLE 1.1 Numbers of lesions on bean hypocotyls treated with 5 % PAE at various times after inoculation with *Colletotrichum lindemuthianum*.

Treatment Time (Hours After Inoculation)	Number of Lesions[a]		Percent Protection[b]
	PAE	Control	
1	49±33	253±82**	81
6	63±34	155±99ns	59
31	300+	300+	0
55	300+	300+	0
79	300+	300+	0

[a] Mean of 4 plants for 1, 6, 31 h treatments and 3 plants for 55, 79 h treatments.
[b] Percent protection calculated as described in Materials and Methods.
** Difference between treatment and control means is significant (t-test, $P < 0.01$).
ns Not significant (t-test, $P > 0.05$).

TABLE 1.2 Development of *Colletotrichum lindemuthianum* on hypocotyls of *Phaseolus vulgaris*. (Stages of fungal development expressed as % of total fungal units observed[a] at each time after inoculation.)

Stages of Fungal Development	Time after Inoculation (Hours)				
	1	6	31	54	78.5
Conidia (ungerminated)	100	51	35	24	52
Germ tubes without appressoria	0	3	0	9	1
Non-pigmented appressoria	0	39	45	0	0
Pigmented appressoria	0	7	20	60	5
Penetration	0	0	0	7	42

[a] Counts made in at least 22 random fields of view (0.25 mm^2) on a single epidermal strip removed from inoculated hypocotyls (ca. 2.5 cm below the cotyledonary node) at stated times after inoculation.

From these data it is concluded that any eradicative action of postinoculation PAE-treatment was limited to preappressorial stages of fungal development on the host tissue surface.

C. Protectant Activity

1. Phaseolus vulgaris - Colletotrichum lindemuthianum.

When a 2 h interval existed between treatment of bean hypocotyls with 5 % PAE and inoculation with C. lindemuthianum, protection was 87 % relative to inoculated controls. The level of protection decreased with increasing dilution of the PAE treatments; dilution endpoint for protection occurred between 0.5 and 1 % PAE (Fig. 1.4). The rate of disease development on plants treated with $\geq$ 1 % PAE was much reduced relative to inoculated controls (sprayed with 5 % ethanol 2 h before inoculation) and plants treated with lower levels of PAE (Fig 1.4).

When PAE-treatment preceded inoculation by intervals ranging from 2 h to 10 d, lesions on hypocotyls were always fewer but usually larger than on inoculated control plants, and complete protection was generally not obtained. With the use of Method I, numbers of lesions on PAE-treated hypocotyls were reduced 74 to 95 % relative to inoculated controls (Table 1.3A). This method required several different inocula and involved plants of different ages - both potential sources of variability in symptom expression.

Fig. 1.4 Rate of lesion development on hypocotyls of *Phaseolus vulgaris* treated with dilutions of PAE 2 h prior to inoculation with *Colletotrichum lindemuthianum*. (Each point is mean of lesion counts on 4 to 8 replicate plants. Percent of control calculated as described in Materials and Methods.)

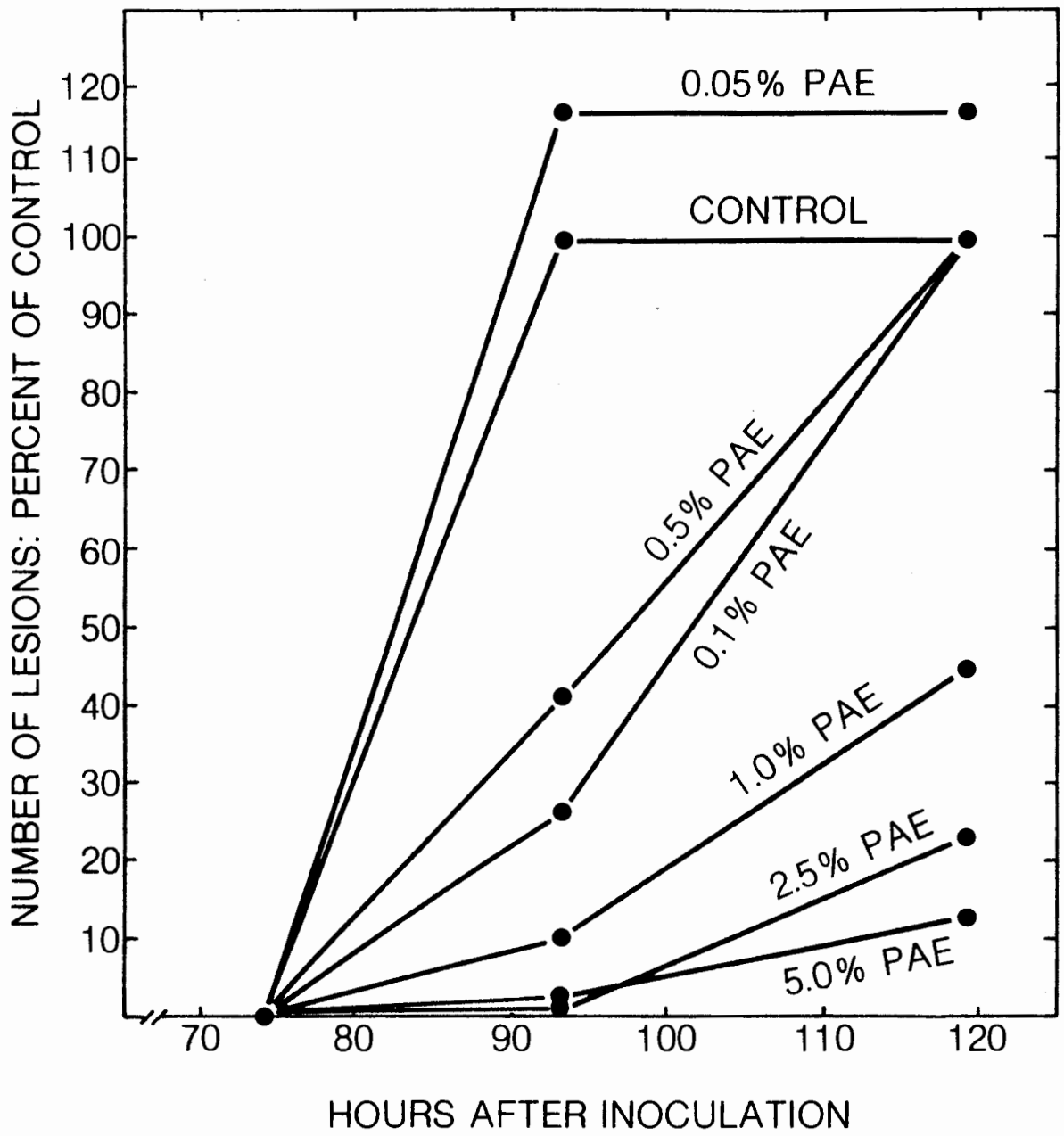

NUMBER OF LESIONS: PERCENT OF CONTROL
120
110
100
90
80
70
60
50
40
30
20
10
0.05% PAE
CONTROL
0.5% PAE
0.1% PAE
1.0% PAE
2.5% PAE
5.0% PAE
70
80
90
100
110
120
HOURS AFTER INOCULATION

TABLE 1.3 Effect of preinoculation PAE-treatment on numbers of lesions occurring on *Phaseolus vulgaris* following inoculation by *Colletotrichum lindemuthianum* using methods I and II[a].

Preinoculation Interval	Age of Plant at Inoculation (days)	Number of Lesions[b] PAE	Number of Lesions[b] Control	Percent Protection
A. Method I				
2 h	10	11	198	95
1 d	11	42	176	76
3 d	13	79	300+	74
4 d	14	74	300+	75
6 d	16	82	300+	73
8 d	18	53	300+	73
10 d	20	8	44	82
B. Method II				
2 h	12	17	300+	94
1 d	12	33	300+	89
3 d	13	18	300+	94
5 d	15	11	150	92
7 d	17	0	50	100
9 d	19	0	15	100
10 d	20	0	16	100

[a] See Materials and Methods for description of test methods.
[b] Mean of lesion counts on 5 to 8 PAE-treated plants and 5 to 16 control plants.
[c] Percent protection calculated as described in Materials and Methods.

With Method II, the reduction in numbers of lesions was 88 to 100 % (Table 1.3B). Although the protection afforded by PAE was reasonably high, all PAE-treated and inoculated plants as well as inoculated control plants eventually died as a result of lesions which developed on their epicotyls. As few as one or two lesions girdling this tissue were sufficient to cause plant death.

A major variable which was uncontrolled by either Method I or II was mature plant resistance (47, 104, 110). This is indicated by the reduced numbers of lesions which occurred on hypocotyls of control plants inoculated when 17 to 20 d old, as compared with plants inoculated when younger (Table 1.3 A and B).

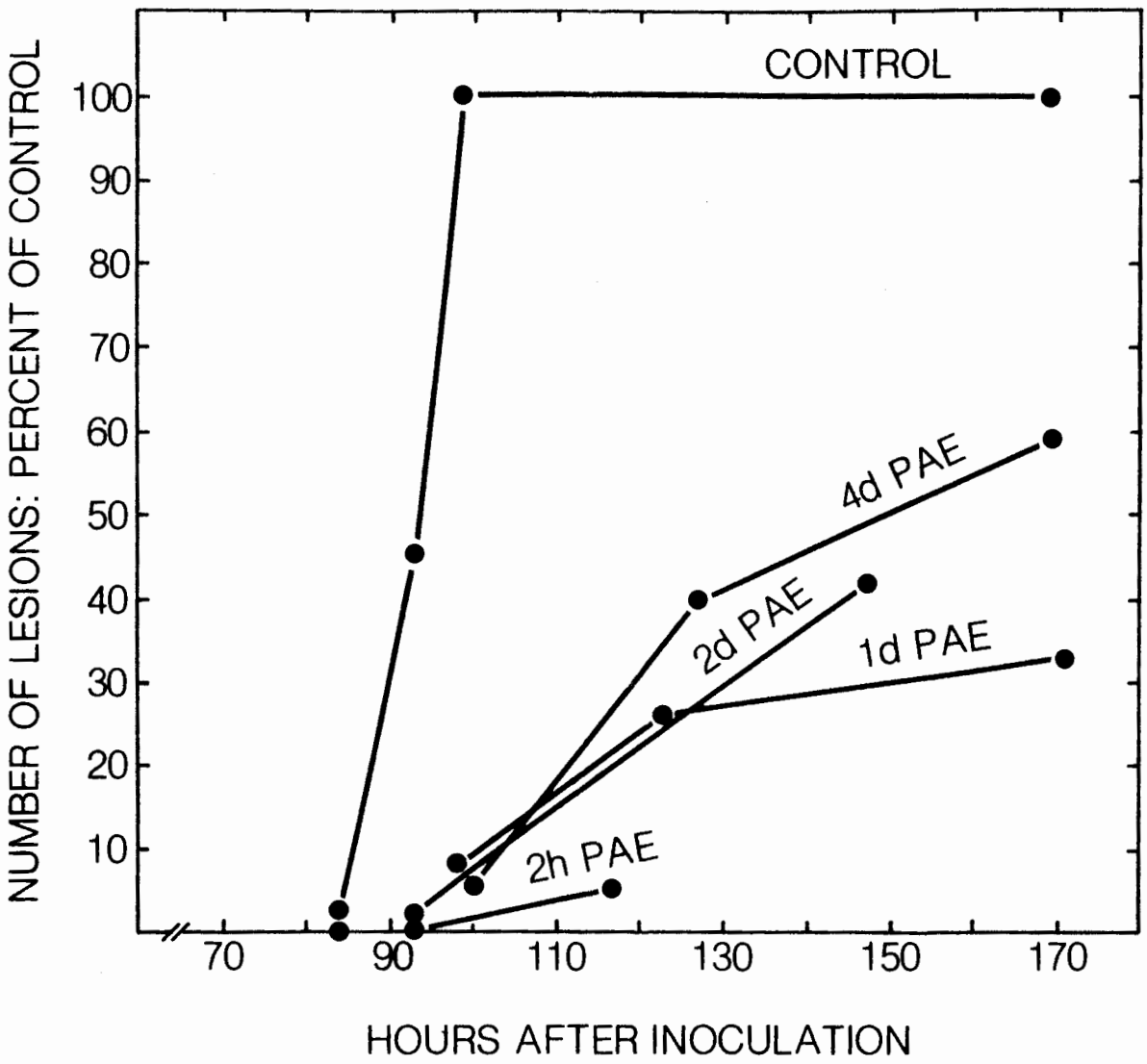
The best control of factors affecting the variability of lesion numbers among replicate control plants was provided by Method III and this method was used for the remainder of the investigation of PAE-persistence on P. vulgaris. Plant protection afforded by PAE using Method III ranged from 96 to 72 % for preinoculation intervals of 2 h to 6 d (Table 1.4). The rate of disease development on all PAE-treated plants was reduced relative to that occurring on inoculated controls (Fig 1.5).

TABLE 1.4 Effect of preinoculation PAE-treatment on numbers of lesions occurring on *Phaseolus vulgaris* following inoculation by *Colletotrichum lindemuthianum* using Method III[a].

Preinoculation Interval	Number of Lesions[b]	Percent Protection[d]
2 h	6	96
1 d	35	79
2 d	37	78
3 d	6	96
3.5 d	20	88
5 d	19	89
6 d	46	72
Control	166[c]	---

[a] See Materials and Methods for description of test method.
[b] Mean of lesion counts on 5 replicate plants treated at each preinoculation interval.
[c] Mean of lesion counts on 34 control plants pooled from all preinoculation intervals.
[d] Percent protection calculated as described in Materials and Methods.

Fig. 1.5 Rate of lesion development on hypocotyls of *Phaseolus vulgaris* treated with 5 % PAE at intervals of 2 h to 4 d prior to inoculation with *Colletotrichum lindemuthianum*. (Each point is mean of lesion counts on 5 to 8 replicate plants. Percent of control calculated as described in Materials and Methods.)


CONTROL
4d PAE
2d PAE
1d PAE
2h PAE
NUMBER OF LESIONS: PERCENT OF CONTROL
100
90
80
70
60
50
40
30
20
10
70
90
110
130
150
170
HOURS AFTER INOCULATION

The addition of Later's spreader-sticker to preinoculation treatments had no effect on the protection afforded by either PAE (5 %) or Captan® (1 ppm) on P. vulgaris inoculated with C. lindemuthianum. There was, likewise, no difference in symptoms between inoculated control plants sprayed with ethanol plus spreader-sticker at preinoculation intervals, and the water-inoculated control plants.

Both PAE-and Captan®-treatments yielded distinct levels of protection against C. lindemuthianum. When plants were grouped into 4 classes related to the level of infection occurring on their hypocotyls, most of the PAE-treated plants fell into class 2 (moderate infection), whereas Captan®-treated plants were found mainly in class 1 (light infection) (Fig. 1.6; Table 1.5).

In summary, the results of the investigation on persistence of PAE-protectant activity on P. vulgaris against C. lindemuthianum showed that protection averaged ca. 75 %. The level of protection was not much influenced by age of plant at the time of PAE-treatment (8 to 14 d), duration of preinoculation interval (2 h to 10 d), plant age at inoculation (10 to 17 d), or presence of an adjuvant (1500, 2500, 3500 ppm).

Fig. 1.6 Classes of infection on 6.5 cm hypocotyl portions of Phaseolus vulgaris inoculated with Colletotrichum lindemuthianum: (a) uninoculated control (0), (b) light infection (1), (c) moderate infection (2), (d) heavy infection (3).

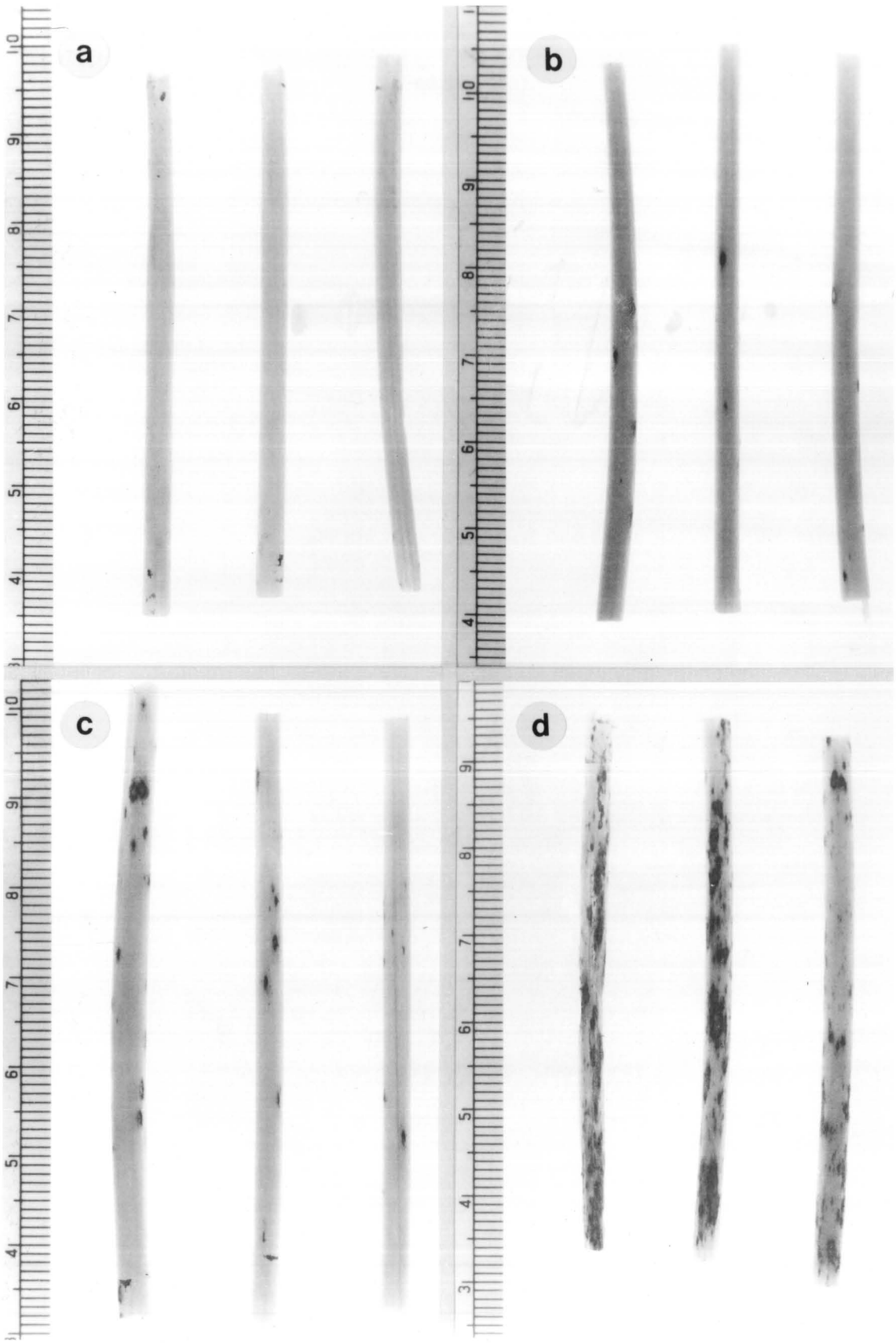

a
b
c
d

TABLE 1.5 Effects of PAE- or Captan®[a]-pretreatments, adjuvant amendments[b] and pretreatment intervals on infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. (Numbers refer to classes[c] of infection and are based on 3 replicate plants.)

Preinoculation Interval (days)	Adjuvant (ppm)	Treatment		
		Control	PAE	Captan®
1	0	ND	2	ND
	1500	3	2	1
	2500	3	2	1
	3500	3	3	3
2	0	ND	2	ND
	1500	3	2	1
	2500	3	2	1
	3500	3	2	2
3	0	ND	2	ND
	1500	3	2	1
	2500	3	2	2
	3500	2	2	1
4	0	ND	2	ND
	1500	3	3	3
	2500	3	2	1
	3500	3	3	1
5	0	ND	1	ND
	1500	3	2	1
	2500	3	2	ND
	3500	2	2	1

[a] N-trichloromethylthiotetrahydrophthalimide (Chevron Chemical Canada, Ltd.).
[b] Later's Spreader-Sticker (Later's Chemicals Ltd., Richmond, B.C.).
[c] Infection classes: 1 = light → 3 = heavy infection.

2. Zea mays - Helminthosporium carbonum. The protective activities of Captan® (1 ppm) and PAE (5 %) with adjuvant were further compared using the corn - H. carbonum host-parasite interaction. No correlation was noted between the amount of dead tissue due to infection and the three adjuvant concentrations (Table 1.6). For both PAE- and Captan®-treated plants, leaf mortality under all treatment conditions was reduced by only ca. 25 % compared with the ethanol-treated inoculated controls, but there was an average 75 % reduction in plant mortality as a result of either PAE- or Captan®-treatment. All corn plants treated with Captan® or PAE at intervals up to and including 1 d before inoculation survived infection with H. carbonum (Table 1.6).

TABLE 1.6 Effects of PAE- or Captan®[a]-pretreatments, adjuvant amendments[b] and pretreatment intervals on infection of Zea mays by Helminthosporium carbonum, expressed as percentages of dead leaves and plants occurring in various treatments[c].

		Treatment					
		Control		PAE		Captan®	
Preinoculation Interval	Adjuvant (ppm)	% leaf mortality	% plant mortality	% leaf mortality	% plant mortality	% leaf mortality	% plant mortality
2 h	0	ND	ND	49.0	0.0	ND	ND
	1500	ND	ND	49.0	0.0	48.1	0.0
	2500	69.0	20.0	54.1	0.0	52.5	0.0
	3500	57.3	0.0	45.4	0.0	52.1	0.0
1 d	0	ND	ND	50.7	0.0	ND	ND
	1500	61.3	20.0	47.3	0.0	ND	ND
	2500	60.1	0.0	44.2	0.0	54.7	0.0
	3500	74.8	40.0	55.5	0.0	54.3	0.0
2 d	0	ND	ND	50.0	0.0	ND	ND
	1500	ND	ND	57.4	0.0	54.7	0.0
	2500	71.0	20.0	71.4	40.0	66.9	20.0
	3500	65.9	0.0	66.8	20.0	59.3	0.0

	0	ND	ND	62.4	40.0	ND	ND
	1500	73.3	40.0	47.0	0.0	ND	ND
3 d	2500	100.0	100.0	59.0	20.0	83.3	60.0
	3500	93.8	75.0	60.7	20.0	49.0	0.0
	0	ND	ND	75.5	20.0	ND	ND
	1500	ND	ND	64.5	20.0	62.5	20.0
4 d	2500	95.5	80.0	45.3	0.0	60.0	20.0
	3500	79.3	40.0	54.3	20.0	58.4	0.0

[a] N-trichloromethylthiotetrahydrophthalimide (Chevron Chemical Canada, Ltd.).
[b] Later's Spreader-Sticker (Later's Chemicals Ltd., Richmond, B.C.).
[c] Percent leaf or plant mortality calculated as described in Materials and Methods for five replicate plants per control or treatment with 5 to 7 leaves each.

DISCUSSION

Suggestions for disease control which have originated from the intensive study of phytoalexins by plant pathologists in the last 20 years include:

1. controlled induction of phytoalexins in plants by fungicides (111), by inoculation of plants with incompatible pathogenic strains (20, 40, 109, 110) and by application of fungal products termed 'elicitors' (2, 151),
2. breeding for monogenic resistance based on production of phytoalexins (100), and
3. application of antifungal plant products as protective fungicides on crops (28, 41, 66, 67, 145, 151).

Following a report of disease control achieved with the use of capsidiol as a protective fungicide (151), I have tested the phytoalexins from P. vulgaris as protectants. Under defined laboratory conditions, protection of selected crop plants against their respective pathogens was obtained. My results, however, do not appear to warrant the same hope for field application of phaseollin and related compounds that Ward and coworkers (151) expressed for capsidiol.

Complete protection of plants inoculated with fungal spore suspensions containing PAE indicates the effective fungitoxic property of this extract when allowed to contact fungal spores.

Microscopic observations of C. lindemuthianum spores from suspensions containing PAE were similar to those of other workers who have reported a disruption of cellular contents and irregular spore shapes after only a 2 min exposure of C. lindemuthianum conidia to 10 µg/ml phaseollin (126).

The dilution endpoint experiments gave information concerning the relative sensitivity of C. lindemuthianum and H. carbonum spores to Captan® and phytoalexins in PAE. Both fungi were more sensitive to Captan® than PAE; H. carbonum was more tolerant to both fungicides than was C. lindemuthianum. Spores of C. lindemuthianum are apparently more sensitive to phaseollin [ED_{50} < 2 ppm, i.e. < 6.25×10^{-6} M; (11)], than are the spores of P. infestans to capsidiol [ED_{50} 4.0×10^{-5} M; (150)]. In addition, the dilution endpoint for fungicidal activity of PAE against C. lindemuthianum (i.e. 12 to 120 ppm) easily falls within the threshold value (ED_{50} < 100 ppm) which is employed for screening potential fungicides (153). Thus one might predict that effective control of bean anthracnose would be achieved with the use of PAE as a protective fungicide.

The data of the persistence studies, do not, however, support this prediction. PAE-treatment-inoculation intervals as short as 2 h reduced protection of P. vulgaris hypocotyls against C. lindemuthianum. It seems that growth occurring

in the epicotyl region of P. vulgaris stems subsequent to fungicide spray-treatment should be unprotected but in contrast to PAE-treated (2 h to 10 d) plants which succumbed to extensive lesion formation on epicotyls, plants pretreated with Captan® as much as 4 d before inoculation with C. lindemuthianum survived and produced flower buds 2 wks after inoculation. The reduction of foliar lesions resulting from PAE-treatment in the corn - H. carbonum interaction also does not represent an adequate level of disease control under the conditions employed, particularly since lesions on corn leaves tend to spread and sporulation from infection sites can take place as early as 48 h after inoculation.

The finding that PAE has postinoculation activity in the P. vulgaris - C. lindemuthianum interaction against ungerminated and germinated spores (preappressorial) is supported by the observations of Skipp and Bailey (127) who noted that for most fungi tested, spores and 1-d old sporelings were equally sensitive to phytoalexins produced by P. vulgaris. Germ tubes of C. lindemuthianum suffered severe disruption when exposed to only 3 ppm phaseollin (13). It may be indirectly concluded from Tables 1.1 and 1.2, that C. lindemuthianum appressoria on P. vulgaris hypocotyls are resistant to any phytoalexins in residual PAE and that hyphal growth subsequent to penetration escaped contact with active compounds in PAE.

Since the effective fungitoxic activity of PAE (5 %) allowed to contact fungal spores has been clearly shown, the concentration of active compounds in PAE applied to plant surfaces must be reduced in some manner, even within 2 h of application. Several possible explanations, involving the host plant, the pathogen and/or the environment, can be offered.

The action of the host plant may involve adsorption of phytoalexin molecules to the nonpolar cuticular surfaces, hydrolysis of compounds through the action of plant excretions or active uptake and/or metabolism of phytoalexins by plant cells. Muller (98) was the first to report that free phytoalexins in diffusates were "fixed" by the epidermal and parenchyma tissue of P. vulgaris bean pods. Other workers (45, 51, 144), have since observed the adsorption of phytoalexins by plant tissue. Skipp and associates (128) reported that over one-half the phaseollin added to a tissue culture of P. vulgaris was taken up by these cells within 24 h; this uptake was associated with plant cell metabolism of phaseollin (45, 51, 128). Furthermore, Phaseolus aureus Roxb. also metabolized phaseollin, although this phytoalexin does not originate in P. aureus (45). Thus, it is possible that plant metabolism of chemical components of PAE affects the persistence of the antifungal activity of this extract on both

corn and bean plants. Although no evidence of systemic action by PAE was seen in this study, phytoalexins in PAE applied to plant surfaces prior to inoculation could conceivably move across the cuticle and become altered by host tissue metabolism.

Fungal mycelia are also capable of passive adsorption (104) as well as absorption and/or metabolism of phytoalexins (145). Colletotrichum lindemuthianum metabolizes phaseollin (24), phaseollinisoflavan (10, 54), phaseollidin (10) and kievitone (10, 129). It is not known whether H. carbonum is able to metabolize the phytoalexins from beans but this fungus cannot metabolize medicarpin (57). A 9 h half-life for phaseollin (15 ppm) in a macroconidial suspension of Fusarium solani (Mart.) Sacc. f. sp. phaseoli (Burk) Snyd. and Hans (56) indicates that germinating spores as well as fungal mycelia metabolize phytoalexins.

The third possibility for the apparent reduction in the fungitoxic activity of PAE on plant surfaces is the influence of environment, specifically light and air. VanEtten and Bateman (143, 144) observed that phaseollin became degraded upon drying on thin-layer chromotographic plates and that exposure to UV light resulted in decomposition of phaseollin. Although phaseollin and related phytoalexins are stable indefinitely in aqueous solution (30), drying of PAE on plant surfaces may cause loss of activity.

For both dilution endpoint and persistence tests, the rate of bean anthracnose disease development on PAE-treated plants was reduced relative to control plants with high density infections (ca. 1.0×10^6 conidia/ml). Several workers have reported both fungicidal and fungistatic modes of action for phaseollin and related compounds depending on their concentration (98), length of time fungi are exposed to active compounds (32, 54) and method of bioassay (10, 12, 126, 127, 144). From the results shown in Fig 1.4, it is possible that between 0.1 to 1 % PAE (12 to 120 ppm) lies a threshold concentration range separating fungistatic and fungicidal effects on C. lindemuthianum spores. In addition, Rahe (108) observed that the appearance of first symptoms in compatible low-density infections of C. lindemuthianum on beans is delayed 20 to 40 h compared with the visualization of symptoms in compatible high-density infections. Therefore, if a fungicidal mechanism is involved in the protection afforded by PAE, a dose-dependent reduction in inoculum would be expected to produce a delay in symptom expression characteristic of low density infections (Fig. 1.4: 1.0, 2.5, 5.0 % PAE and Fig. 1.5). On the other hand, a fungistatic mechanism would delay spore germination on plant surfaces but would allow eventual symptom expression comparable to controls (Fig. 1.4: 0.1, 0.5 % PAE).

Protective fungicides have three essential characteristics in common (117):

1. biological activity,
2. solubility characteristics which allow the compounds to be taken up by a pathogen in toxic amounts, and
3. adequate residuality following application.

Reports on the use of natural plant products with antifungal properties as protective fungicides have contained both successes and failures. Wyerone, beta-thujaplicin and juglone all gave nearly 100 % control of rust on beans while vanillin, protocatechuic acid and gallic acid were used as protectants against Piricularia oryzae on rice (41). On the other hand, Harris and Dennis (53) observed no significant control of P. infestans on potato, Uromyces fabae (Pers.) de Bary and Botrytis fabae Sardiña on broad bean, or Erysiphe graminis De Candolle on wheat, when metabolites of potato (e.g. rishitin, phytuberin) were used as protectant sprays at 100 ppm.

From this study of PAE involving 4 disease interactions, I conclude that the phytoalexins of beans are only moderately effective as broad spectrum protective fungicides due to their lack of persistence on plant surfaces. In addition, they have only minor eradicative properties by assessment with the bean anthracnose fungus. The only possibilities which remain for

practical use of these antifungal compounds as protectants would be in the synthesis of analogues having greater stability on plant surfaces or in the control of a specific disease or class of fungi not yet tested. With respect to the latter possibility, no report has been published regarding the usefulness of capsidiol as a protectant in disease interactions other than tomato blight involving the fungus, P. infestans.

The present investigation may provide a useful contribution to fungicide screening methodology, if not to the reservoir of natural plant product fungicides. Skipp and Bailey (127) reported that in testing the response of C. lindemuthianum to phaseollin, the most consistent results were obtained with young inocula in liquid media. The 'inoculum plus fungitoxicant' bioassay integrates the strengths of other fungitoxicity tests, i.e. the precision characteristic of laboratory spore germination tests (4, 153) and some of the predictive value for field performance characteristic of 'greenhouse' evaluations (93, 153). Furthermore, ED_{50} determinations derived from presently utilized antifungal tests can be inaccurate. Unrealistically low ED_{50} values may result from spore germination tests because of the settling of insoluble test compounds (5). On the other hand, abnormally high ED_{50} values may result from mycelial radial growth tests on agar media due to adsorption of test compounds to agar

particles or localized depletion of a test compound by way of fungal metabolism (12). With the use of the 'inoculum plus fungitoxicant' bioassay, these problems are avoided because inoculation immediately follows spore contact with the ambient concentration of a test compound. Poor correlation between in vitro and 'greenhouse' evaluations of fungicides have resulted when compounds with apparent toxicity in laboratory tests failed to provide an acceptable level of disease control (153). Differences in fungistatic and fungicidal modes of action would be revealed in the above bioassay. Finally, successful use of this bioassay for screening potential foliar fungicides would be dependent on the choice of disease interactions resulting in discrete symptoms (e.g. lesions) which are easily quantified and directly dependent on fungal spore concentration (93).

CHAPTER 2

EVALUATION OF PLANT NATURAL PRODUCTS AS INSECT REPELLENTS AND/OR DETERRENTS

INTRODUCTION

Laboratory studies investigating the potential repellency of phytoalexins and other plant natural products involved the onion maggot, Hylemya antiqua Meig. This Dipteran insect was selected firstly, because the behavioural responses of H. antiqua to its host, Allium cepa L. (onion) are strongly directional (89, 90, 147) and secondly, because the onion maggot is relatively easy to rear.

The effects of various organic sulfur compounds on H. antiqua oviposition have been studied by Matsumoto and Thorsteinson (90) who found that n-propyl disulfide and n-propyl mercaptan at certain concentrations stimulated increased activity and landing of gravid females and extension and probing activity with proboscis and ovipositor. These same compounds along with methyl disulfide, another component of onion odours, induced orientation and aggregation in maggots (91). N-propyl disulfide and n-propyl mercaptan attracted gravid female H. antiqua in the field and dipropyl disulfide was attractive to both sexes (89).

Additional attractants and oviposition stimulants for H. antiqua have recently been isolated (103, 148). The dual-choice oviposition system which was developed for bioassay of these attractants (147) was adapted to serve as the basic tool in this repellency study.

The early results of my investigation indicated that PAE (phytoalexin extract) possessed repellent activity, but this activity was subsequently found to be unrelated to the presence of phytoalexins in PAE. At this point, a decision was made to expand the original goal of this research to include an examination of oviposition deterrents to H. antiqua found in the tissue extracts of plant species from four different botanical families.

MATERIALS AND METHODS

A. Preparation of Extracts

An extract containing phytoalexins (i.e. PAE) was prepared as described previously (p.5). Several bean seed extracts not containing phytoalexins (BSE_1, BSE_2, BSE_3, BSE_4) were prepared similarly from uninoculated hydrated seed pieces of P. vulgaris 'Topcrop'. A solvent control (SC) was prepared by applying the complete fractionation procedure to a solution of acetone and tap water in proportions representative of those in the initial combined acetone extracts of bean seed pieces. All solvents were reagent grade and were utilized without further purification. Extracts of other tissues of P. vulgaris and of other plant species were also evaluated. These extracts were prepared in a manner similar to that described for hydrated seeds of P. vulgaris. All final test extracts contained extractives at a concentration equivalent to 12 g fresh wt of tissue/ml 95 % ethanol.

B. Choice Bioassays

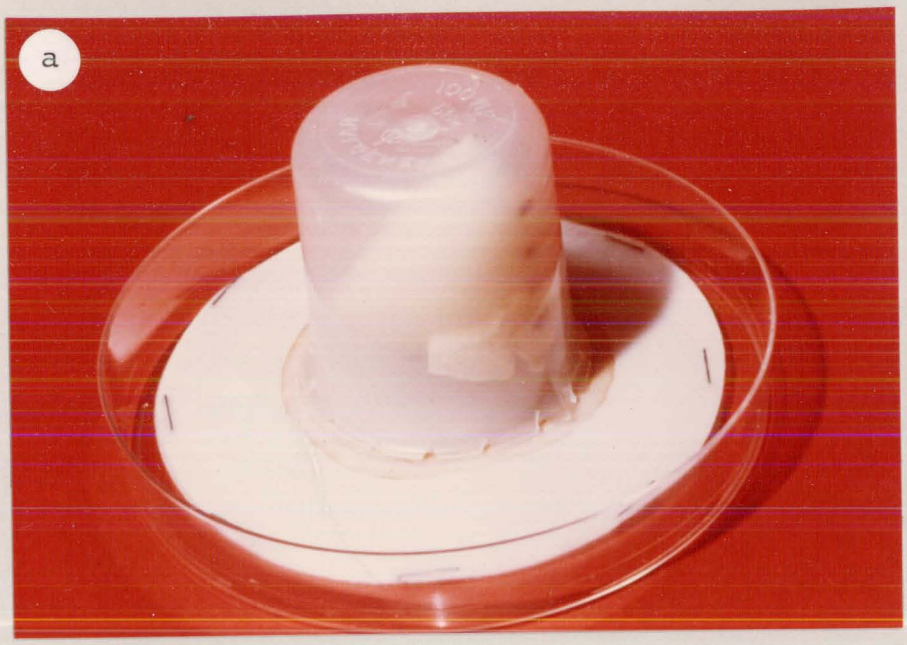
Extracts were evaluated for their ability to deter the oviposition response of H. antiqua to volatile attractants released by cut pieces of mature onion bulbs in a dual-choice oviposition station bioassay. An oviposition station (Fig. 2.1a) consists of an attractant source (in this study, 1/4 of a mature onion bulb, variety unknown) taped inside an autoclaved

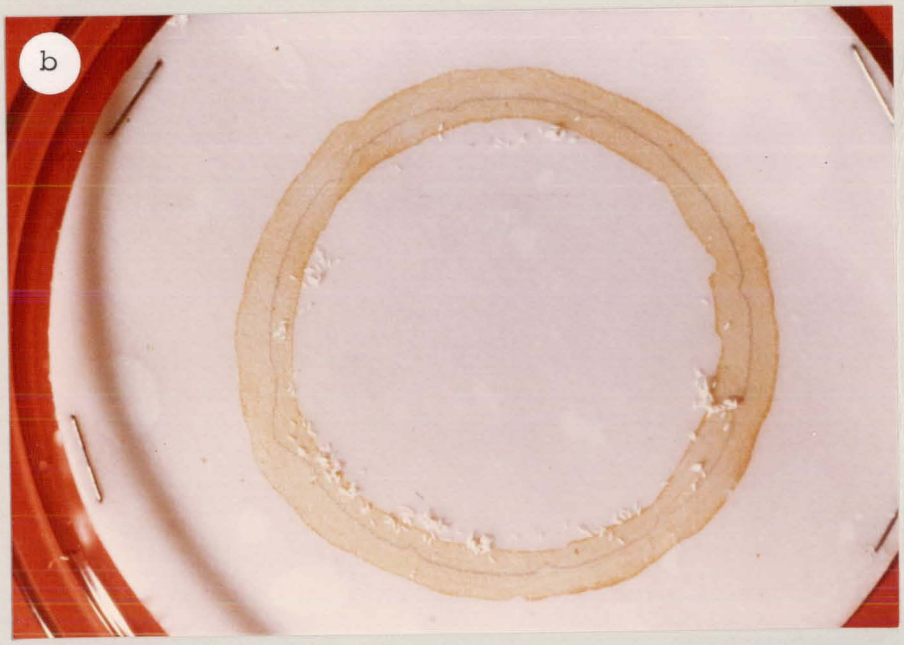
100-ml Nalgene beaker with 12 to 15 small, equally spaced notches cut in its rim (6.4 cm diam). The beaker is inverted onto a waxed disc, 12.5 cm diam, made from 5 Whatman No. 1 filter papers stapled together and soaked in molten Parowax (Imperial Oil, Ltd.). This apparatus is placed in a sterile petri dish lid (14 cm diam) and positioned in a cage with gravid female flies. The flies deposit eggs singly or in clusters on the waxed disc just inside the notches of the inverted beaker (Fig. 2.1b).

To bioassay for deterrent activity 100 ul of a plant extract was applied uniformly to the waxed filter paper in a ring ca. 9 mm wide centered on an outline of the rim of the Nalgene beaker. Tissue extracts were thus presented to test insects at a concentration equivalent to 74.6 mg fresh wt of tissue per cm^2 at the sites of oviposition. The complete solvent control (SC) was applied similarly and provided any solvent effects or impurities in the same concentrations as would be present in the tissue extracts. Extracts were assigned deterrent activity when the number of eggs laid near the host in the presence of an extract was significantly reduced relative to the normal oviposition response of gravid female H. antiqua to onion volatiles at the control oviposition site.

Rearing procedures for H. antiqua and the nature of cages and environmental conditions for bioassays were identical

Fig. 2.1 Oviposition station (a) and eggs laid by *Hylemya antiqua* (b) in response to volatile chemicals released from onion piece within beaker.


a


b

to those described by Vernon *et al.* (147). Bioassays were carried out in controlled environment chambers with 8 or 10 replicate cages. Except where otherwise stated, each cage contained 15 gravid female flies, food dish, water dish, and 2 oviposition stations - one with test extract and the other with control extract or solvent. A bioassay period of 3 to 4 d was chosen because the numbers of eggs laid by *H. antiqua* was typically high and low on consecutive days. Eggs were counted and removed every 24 h. The food and water dishes in each cage were placed right-rear and left-front, respectively; control and test stations were placed left-rear and right-front and their relative positions were reversed in alternative cages. Each experiment was preceded by a 24-h period in which the flies were held in bioassay cages with food and water only, to prepare them for oviposition.

Since insects respond to chemical stimuli via olfactory (volatile) or gustatory (contact) mechanisms, these alternatives were investigated by comparing the deterrency of extracts applied as a ring centered under the rim of the beaker (contact mode) with that when applied as an inaccessible centrally-located spot beneath the beaker (volatile mode). The total amounts and concentrations of extract/cm^2 of treated surface area on the waxed discs were identical for the two modes.

Some bioassays consisted of 3 oviposition stations (3 treatment tests). For these, waxed discs of 9 cm diam and petri dish lids of 10 cm diam were used, but the size of the Nalgene beakers was the same as used for dual-choice experiments. The 3 treatments were positioned at the points approximating an equilateral triangle within each test cage, and their positions were randomized in the replicate cages.

The mean number of eggs per female per day at each treatment in each cage was determined. Their numbers were analyzed using analysis of variance programme BMD08V (115). The Newman-Keul's test (155) was used for multiple comparisons.

C. Mass Rearing Cage Bioassays

Oviposition stations prepared as described above were placed in a rearing cage containing 150 to 300 gravid female H. antiqua. Temperature and light conditions were consistent with those used for rearing H. antiqua, i.e. ca. 23 C and 16:8 LD regime. Eggs were counted and removed every 24 h. This bioassay was used for screening tests involving 3 or more materials, for evaluation of the persistence of deterrent activity in extracts applied to oviposition stations, and for study of concentration effects on deterrent activity.

D. Simulated Field Trials

Onion seeds (A. cepa 'Autumn Spice') were planted in muck soil in plastic trays (12.5 x 16.75 x 5.5 cm). The soil was kept moist and seedlings were thinned to 48 per tray on the 12th d after seeding. Five ml of an extract from etiolated hypocotyls of 7-d-old P. vulgaris 'Topcrop' seedlings was then sprayed uniformly with an atomizer onto the seedlings and soil surface in 2 trays. Control trays were sprayed with an equivalent amount of 95 % ethanol.

Two treated and two control trays were placed in diagonally opposite corners of a holding tray (26 x 52 cm); food and water dishes (as described in the dual-choice bioassay) were placed on a platform between the seedling trays. The holding tray was then placed in a mass rearing cage and exposed to varying numbers of gravid females for varying time periods.

Following exposure to H. antiqua, the seedlings were maintained under the same environmental conditions as above. Soil moisture was maintained by watering from the bottoms of the trays. At the first sign of maggot damage, the trays were moved to the laboratory bench (RT; normal laboratory lighting). Numbers of fallen seedlings per tray were recorded daily.

RESULTS

A. Occurrence of Oviposition Deterrents in Hydrated Seeds of Phaseolus vulgaris

Oviposition by H. antiqua was deterred significantly by PAE in two trials (Table 2.2, Exp. 1). To obtain further evidence as to whether phytoalexins were the deterrent principle(s) of PAE, I next tested PAE against an extract of healthy bean seeds (BSE_1). Surprisingly, BSE_1 was significantly more deterrent than PAE (Table 2.2, Exp. 2). This experiment was repeated twice with BSE_3 and each time BSE was equally or more deterrent than PAE. Comparative chemical analyses of PAE and BSE_1 for phytoalexins (as described previously, p.6), indicated that any amounts of phytoalexins present in the BSE's would have been less than 1 % of the corresponding levels present in PAE (i.e. 3.0, 3.3, and 5.6 ug/ul for phaseollin, phaseollinisoflavan, and kievitone, respectively). Apparently the phytoalexins were not the deterrent principle(s).

In Experiments 3 to 6 (Table 2.2) I tested various BSE's against ethanol and/or the complete solvent control (SC). In each case, oviposition was deterred significantly by the BSE's. Results of a triple-choice bioassay testing BSE_3, SC and a 3rd station containing only the onion piece attractant source (OC) provided further indication that the solvents used to prepare

TABLE 2.1 Summary of abbreviations used in data tables to denote plant extract treatments.

Extract	Description
PAE	phytoalexin extract
$BSE_{1,2,3,4}$	bean seed extracts
SC	solvent control
OC	onion control
OT	extract of onion leaves
OB	extract of onion bulbs
BSE_3vol, OT_{vol}, OB_{vol}	BSE_3, OT or OB in volatile mode
PM	extract of P. vulgaris 'Perry Marrow'
grass I	extract of velvet grass
grass II	extract of Kentucky bluegrass
dye-control	Nabob food-colouring dye plus 95% ethanol
TC-21-d	extract of 21-d-old light-grown P. vulgaris 'Topcrop'
TC-15-d	extract of 15-d-old light-grown P. vulgaris 'Topcrop'
TC-5-d	extract of 5-d-old etiolated P. vulgaris 'Topcrop'
TC-7-d	extract of 7-d-old etiolated P. vulgaris 'Topcrop'

TABLE 2.2 Effect of extracts from seeds of Phaseolus vulgaris 'Topcrop' on oviposition rates of Hylemya antiqua in dual- and triple-choice bioassays.

Experiment	Duration (Days)	Treatment choices[a]	Oviposition rate[b]
1	4	PAE	3.58**
		95% ethanol	9.59
2	4	BSE_1	2.35**
		PAE	10.21
3	4	BSE_1	2.56**
		95% ethanol	10.33
4	4	BSE_2	1.22**
		95% ethanol	11.04
5	4	BSE_2	1.90*
		SC	4.47
6	4	BSE_3	2.71**
		SC	8.30
7	4	BSE_3	0.70[a]**
		OC	3.44[b]
		SC	4.43[b]
8	4	OC	4.97[ns]
		SC	10.65
9	3	BSE_3 con	1.12[a]*
		BSE_3 vol	4.93[b]
		OC	3.49[ab]
10	4	BSE_3 con	3.61**
		BSE_3 vol	8.63

[a] See Table 2.1 for explanation of various treatments.
[b] Mean number of eggs/female/treatment/day. Significance of lesser rate in dual-choice bioassay indicated by * = $P < 0.05$, ** = $P < 0.01$, ns = not significant (F test, ANOVA). In triple-choice bioassays, rates followed by same letter are not significantly different at * = $P < 0.05$, ** = $P < 0.01$ (Newman Keul's Test).

BSE were not deterrent (Table 2.2, Exp. 7); treatment effect was significant only for BSE_3. Solvent control was subsequently tested in a dual-choice bioassay against OC (Table 2.2, Exp. 8). A greater number of eggs was laid at the station containing SC, but the difference was not significant at $P < 0.05$. I concluded that deterrent activity was contributed by one or more components extracted from bean seeds.

When the mechanism of deterrent activity in BSE was examined, BSE was significantly deterrent only when applied in a manner that allowed H. antiqua to come into physical contact (i.e. contact mode) with the treated portion of the waxed disc (Table 2.2, Exp. 9 and 10).

B. Range of Occurrence of Oviposition Deterrents for Hylemya antiqua

To test whether the deterrent principle(s) was (were) limited to hydrated seeds of P. vulgaris 'Topcrop', I prepared extracts of hydrated seeds of Perry Marrow, a white-seeded cultivar of P. vulgaris, and of soybeans (Glycine max (L.) Merr. 'Altona'). Mass rearing cage and dual-choice bioassays revealed that both extracts were significantly deterrent relative to SC at $P < 0.01$ (Table 2.3, Exp. 1 to 3; Table 2.4, Exp. 1 and 2).

TABLE 2.3 Occurrence of oviposition deterrents to Hylemya antiqua in plant extracts: effect on oviposition in mass rearing cage bioassays.

Experiment	Duration (Days)	Treatment choices[a]	Eggs laid[b]
1	2	PM:SC	425:3300
	2	PM:SC	620:3000
2	1	PM:SC	250:3200
3	1	soybean:SC	490:1800
4	1	TC-5-d:SC	130:3750
	1	TC-5-d:SC	70:1000
5	1	TC-7-d:SC	60:3100
	1	TC-7-d:SC	550:4600
6	1	TC-21-d:grassI:dye-control	10:25:1300
	1	TC-21-d:grassI:dye-control	20:15:985
7	1	dye-control:OC	1400:3300
8	1	OB:OT	500:3000
	4	OB:OT	1300:4000

[a] See Table 2.1 for explanation of various treatments.
[b] Total eggs laid by 150 to 300 gravid females at designated oviposition station in a mass rearing cage for single days or 2-d (Exp. 1) or 4-d (Exp. 8) periods.

TABLE 2.4 Occurrence of oviposition deterrents in plant extracts: effect on oviposition rates of *Hylemya antiqua* in dual- and triple-choice bioassays.

Experiment	Duration (Days)	Treatment choices[a]	Oviposition rate[b]
1	3	PM	1.78**
		SC	19.83
2	3	soybean	4.23**
		SC	15.87
3	3	grass I	2.16**
		dye-control	14.99
4	3	grass II	1.39**
		dye-control	14.65
5	4	TC-21-d	1.28**
		dye-control	13.62
6	4	TC-15-d	1.18**
		dye-control	12.16
7	4	tomato	1.69**
		dye-control	14.13
8	4	OT	2.16a**
		OB	1.51a**
		OC	4.91b
9	1	OT_{vol} (no onion)	0.00a**
		OB_{vol} (no onion)	0.00a**
		OC	6.23b
10	3	OT_{vol} (no onion)	0.01a**
		OB_{vol} (no onion)	0.00a**
		OC	10.82b
11	3	OB vol	6.63
		OC	5.45ns
12	3	garlic	0.96**
		SC	14.82

[a] See Table 2.1 for explanation of various treatments.
[b] Mean number of eggs/female/treatment/day. Significance of lesser rate in dual-choice bioassay indicated by * = $P < 0.05$, ** = $P < 0.01$, ns = not significant (F test, ANOVA). In triple-choice bioassays, rates followed by same letter are not significantly different at * = $P < 0.05$, ** = $P < 0.01$ (Newman Keul's test).

Extracts of other tissues of P. vulgaris at various stages and conditions of development were prepared to find out whether the deterrent(s) was (were) limited to seed extracts of P. vulgaris. The following tissues were included:

1. entire etiolated 'Topcrop' seedlings harvested at 5 to 7 d after hydration of seeds, at which time seedlings measured 9.5 to 11.4 cm and 17.8 to 25.3 cm long, respectively, and
2. entire 15- and 21-d-old light-grown plants with normal chlorophyll development.

These extracts were tested by mass rearing cage bioassay. Extracts from etiolated seedlings of both ages were deterrent when compared with SC (Table 2.3, Exp. 4 and 5), as were the extracts from 15- and 21-d-old light-grown plants. The comparisons regarding light-grown plants require further explanation, however.

Extracts of hydrated seeds or etiolated seedlings were pale yellow in colour such that test extract- and SC-treated areas of the waxed discs were virtually indistinguishable from each other to the human eye. Extracts from light-grown plants were dark green, however, and I attempted to account for this new variable by the use of green-coloured controls in the bioassays. Two types of controls were chosen: green food colouring dye (Nabob Foods Co., Vancouver) and extracts of two

grasses: I = Holcus lanatus L. (velvet grass); II = Poa pratensis L. (Kentucky bluegrass). A screening test (in the mass rearing cage) indicated that both grass extract I and a light-grown plant extract (21-d-old) had deterrent activity compared with the food-colouring dye-control (Table 2.3, Exp. 6). The two grass extracts and the two light-grown bean plant extracts were then tested by the 10-cage dual-choice bioassay. In every case deterrency was significant relative to a dye-control (Table 2.4, Exp. 3 to 6). When the dye-control was tested by mass rearing cage bioassay against OC, there was an equal or fewer number of eggs on the dye-treated disc relative to OC, indicating that the dye was not acting as an attractant, and therefore was a legitimate substitute control for SC (Table 2.3, Exp. 7).

Having established that oviposition deterrent activity to H. antiqua occurs in extracts of various tissues of P. vulgaris, hydrated seeds of another legume, Glycine max, and the monocots: Holcus lanatus L. and Poa pratensis L., I prepared and tested an extract of stems and leaves from 10-wk-old tomato plants (Lycopersicon esculentum Mill. 'Bonny Best'). This extract was also significantly deterrent ($P < 0.01$) when compared with a dye-control (Table 2.4, Exp. 7).

In an effort to find a plant tissue without deterrent activity, I prepared separate extracts of bulbs and foliage of

onion, the host plant for H. antiqua oviposition. When the onion extracts were tested against OC, significant deterrent activity was found in both extracts (Table 2.4, Exp. 8; $P < 0.01$). The extract of onion bulbs had more deterrent activity than the onion foliage extract (Table 2.3, Exp. 8). Bioassay of the onion bulb and onion foliage extracts in the volatile mode without onion piece attractant source demonstrated an absence of volatile attractants or oviposition stimulants in these extracts (Table 2.4, Exp. 9 and 10). No volatile oviposition deterrents were detected in onion bulb extract when it was applied in the volatile mode with an onion piece attractant source and compared to OC (Table 2.4, Exp. 11).

An extract of garlic, A. sativum L., a less-frequented oviposition host for H. antiqua (90), was prepared and tested against SC. Deterrent activity in the garlic extract was significant at $P < 0.01$ (Table 2.4, Exp. 12).

C. Some Aspects of the Nature of Oviposition Deterrent Activity in Extracts of Hydrated Bean Seeds

1. Concentration Effects. The possible effect of concentration on the behavioural response of H. antiqua to deterrent principles in BSE's was tested. Oviposition stations containing BSE_4 in a range of dilutions were placed in a mass rearing cage for 24 h, after which the eggs laid at each station

were counted. The number of eggs laid at each station (mean ± standard error of 4 separate trials, each with a different culture of flies aged 14 to 36 d post-emergence) is shown in Fig. 2.2. At a dilution of 10^4, BSE_4 stimulated oviposition significantly ($P < 0.05$) compared with SC and BSE_4 at dilutions up to 10^2, according to Duncan's Multiple Range Test.

2. Persistence. When gravid female flies in a rearing cage were presented with fresh BSE_4- and SC-treated oviposition stations daily for several consecutive days in a dual-choice situation, the proportion of eggs laid at the BSE_4-treated station was consistently less than that at the SC-treated station (Table 2.5, Exp. 1). The effect of treatment in a no-choice situation where treatments were also replenished daily was tested in Experiment 2, Table 2.5. Five replicate cages of the type used in dual-choice bioassays were used to test insect response to a SC station and another 5 cages in a separate chamber were used to test insect response to the BSE_4 treatment. Gravid females withheld a large proportion of their eggs from the BSE_4 station for the 4-d duration of the experiment when no alternative was available. If, however, the BSE_4 treatment disc alone was presented without replenishing to a H. antiqua culture, deterrency appeared to break down after the second day.

Fig. 2.2 Numbers of eggs laid by *Hylemya antiqua* at oviposition stations treated with various dilutions of an extract of hydrated seeds of *Phaseolus vulgaris*. (Vertical bars indicate standard error of mean for 4 trials, each with a different culture of *Hylemya antiqua*).

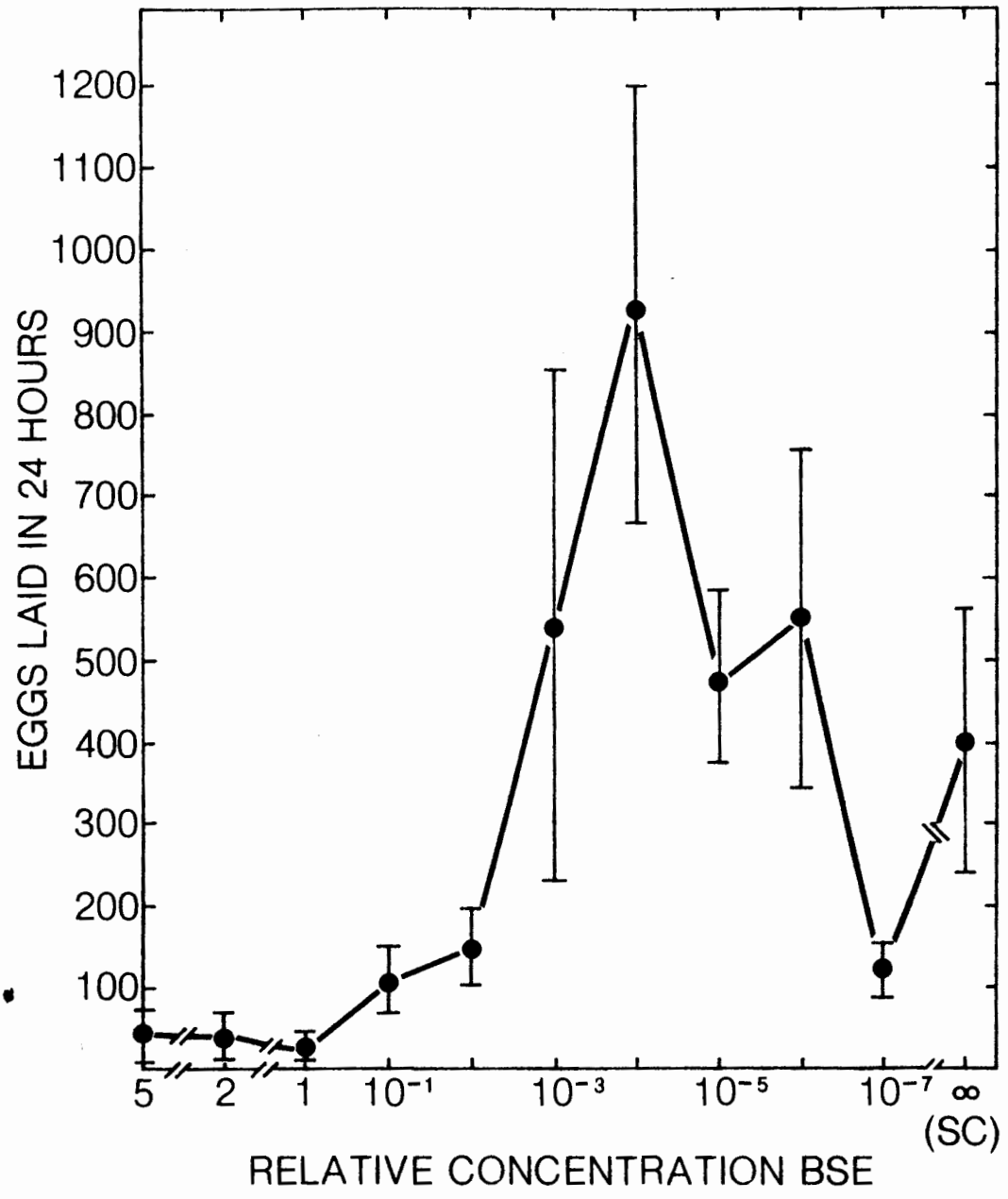

EGGS LAID IN 24 HOURS
1200
1100
1000
900
800
700
600
500
400
300
200
100
5
2
1
10^{-1}
10^{-3}
10^{-5}
10^{-7}
∞
(SC)
RELATIVE CONCENTRATION BSE

TABLE 2.5 Persistence of oviposition deterrent activity of an extract of *Phaseolus vulgaris* seeds to *Hylemya antiqua*.

Day	Eggs laid[a]	
	BSE_4[b]	SC[b]
Experiment 1 (dual-choice)		
1	156	940
2	31	372
3	120	502
4	10	553
5	46	38
6 and 7	10	778
Experiment 2 (no-choice)		
1	71	325
2	239	224
3	162	303
4	51	393

[a] In Experiment 1, total eggs laid by 150 to 300 gravid females at designated oviposition sites in mass rearing cage bioassay; in Experiment 2, total eggs laid in 5 cages each with BSE_4-treated oviposition station or 5 cages each with SC-treated oviposition station. (Each cage contained 15 gravid *H. antiqua*.)

[b] See Table 2.1 for explanation of various treatments.

D. Activity of Extracts Containing Oviposition Deterrents when Applied Directly to Hylemya antiqua Host Tissue

1. Onion Bulb Assay. Potential use of oviposition-deterring extracts for field control of H. antiqua would require the direct application of extracts to the host tissue of H. antiqua. This was tested by applying plant extracts to peeled, scored onion bulbs since Vernon (146) had shown such tissue to be attractive to ovipositing H. antiqua in the field. Medium-sized bulbs (variety unknown) were peeled and cut in half. The bulb halves were placed cut side down in individual petri dishes, scored and treated with plant extract or ethanol. All exposed tissue including scored grooves and the surrounding 2 cm of petri dish floor were "painted" with plant extract or ethanol. Petri dishes with onion pieces were than placed in a mass rearing cage and exposed to a culture of H. antiqua containing $\geq$ 150 gravid females.

In the first experiment, bean extract-painted (ca. 2 ml of 7-d-old etiolated P. vulgaris 'Topcrop' extract) and ethanol-painted onion pieces (control) were compared. Treatment and control dishes were placed on a yellow cardboard square to aid in visual attraction of flies (146). Oviposition occurred in the scored grooves of the onion surface, underneath the onion piece along its periphery, and beside the onion piece on the

petri dish. Egg counts made at the end of each 24-h period for 3 d revealed a consistently reduced egg number in the treatment dish when compared with the control dish (Table 2.6, Exp. 1).

The above experiment was repeated using onion bulb extract (OB) in place of bean extract. The amount of OB which was applied contained extractives equivalent to 60 g fresh wt bulb tissue; bulb halves receiving this treatment weighed 40 to 45 g fresh wt. Figure 2.3a shows the effect of OB on the distribution of flies in the test 1 h after exposure to the treated and control onion dishes. Oviposition was markedly reduced on the onion bulb halves treated with extractives from onion bulbs, compared with bulb halves treated with ethanol (Fig. 2.3b, c, d, e and Table 2.6, Exp. 2).

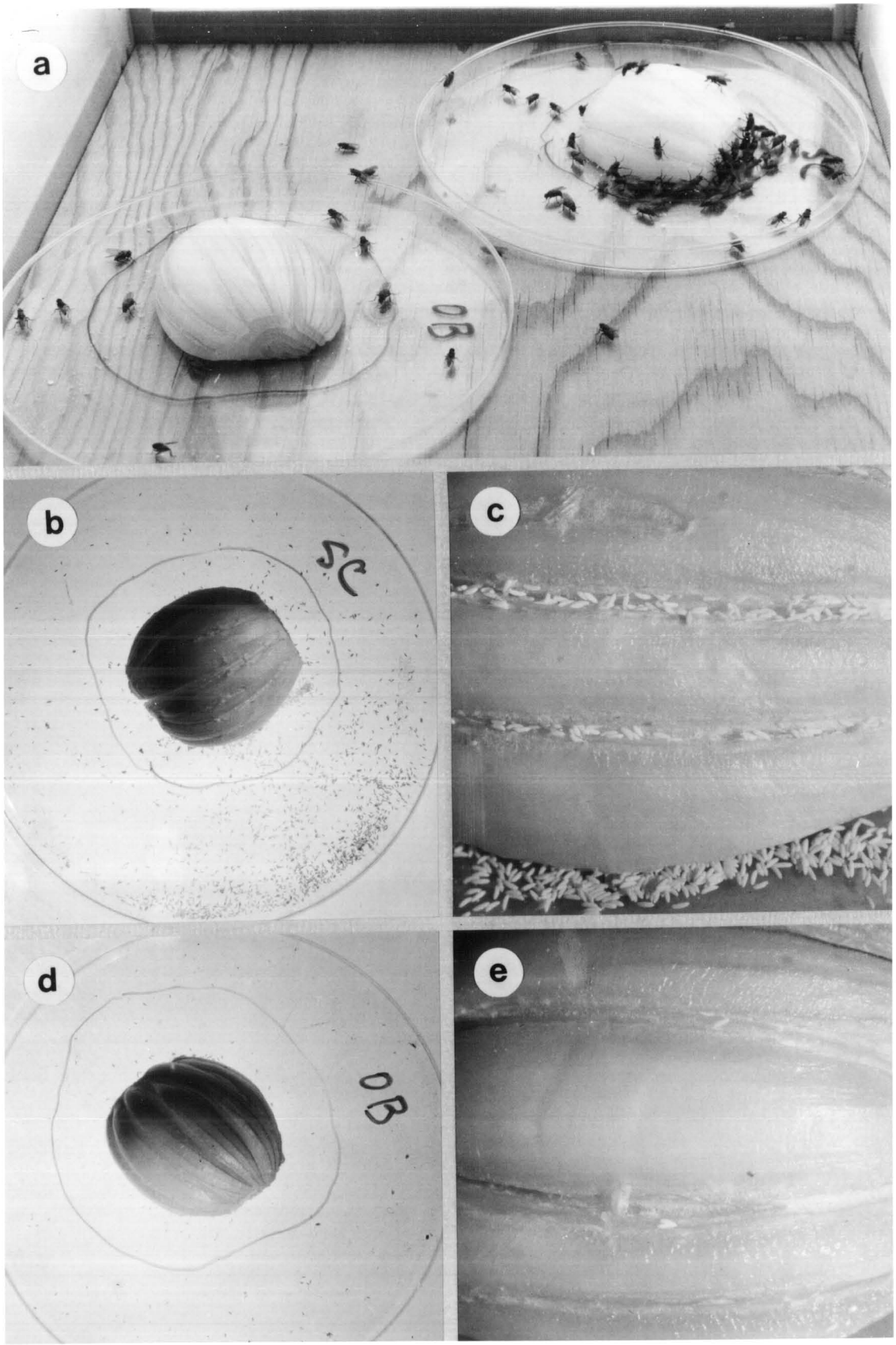
2. Simulated Field Trials. The protective effect of plant extracts containing oviposition deterrents to H. antiqua was evaluated on onion seedlings growing in soil. Plants grown in trays and sprayed as described in the Materials and Methods were presented to mixed cultures of H. antiqua (ca. 150 females) in mass rearing cages for varying periods of time (9.5 to 48 h). Eggs were laid on or just under the moist soil surface at the base of the plants and within 2 to 3 d, hatched larvae had begun to feed on the seedlings just below the soil surface. Seedling death caused by maggot feeding between 2 and 6 d after removal of trays from rearing cages was reduced

TABLE 2.6 Effect of plant extracts[a] on the numbers of eggs laid by Hylemya antiqua at onion bulb oviposition sites.

Experiment	Day	Eggs laid[b]	
		Extract	Control
1	1	40	460
	2	250	635
	3	380	760
2	1	940	9700
	2	420	6700
	3	2800	5200

[a] In Experiment 1, extract of 7-d-old etiolated P. vulgaris 'Topcrop'; in Experiment 2, extract of onion bulbs.
[b] Total eggs laid by 150 to 300 gravid females in a mass rearing cage on consecutive days.

Fig. 2.3 Behavioural responses of *Hylemya antiqua* to *Allium cepa* painted with onion extract: (a) distribution of flies 1 h after their exposure to extract-treated and solvent control (SC)-treated bulbs, (b) and (c) eggs laid on SC-treated bulb, (d) and (e) eggs laid on extract-treated bulb.


a
OB
b
SC
c
d
OB
e

ca. 25 % in the extract-treated replicates relative to the ethanol-treated controls. Damage in the former trays however, eventually reached 100 %. In a separate test I found that a single H. antiqua maggot placed in the center of a tray of 2-wk-old onion seedlings destroyed half of the plants within 19 d (Fig. 2.4).

The maximum level of protection afforded by plant extracts containing oviposition deterrents was obtained when pretreatment was followed by exposure of onion seedlings to ca. 150 female H. antiqua for 40 min (Exp. 2, Fig. 2.5). When only 3 gravid females were used for an exposure time of 48 h (Exp. 3, Fig. 2.5), a reversed outcome was observed.

Fig. 2.4 Rate of mortality of onion seedlings due to feeding by a solitary larva per tray of plants. (Vertical bars indicate standard error of mean of 4 replicate tests for each time of observation).

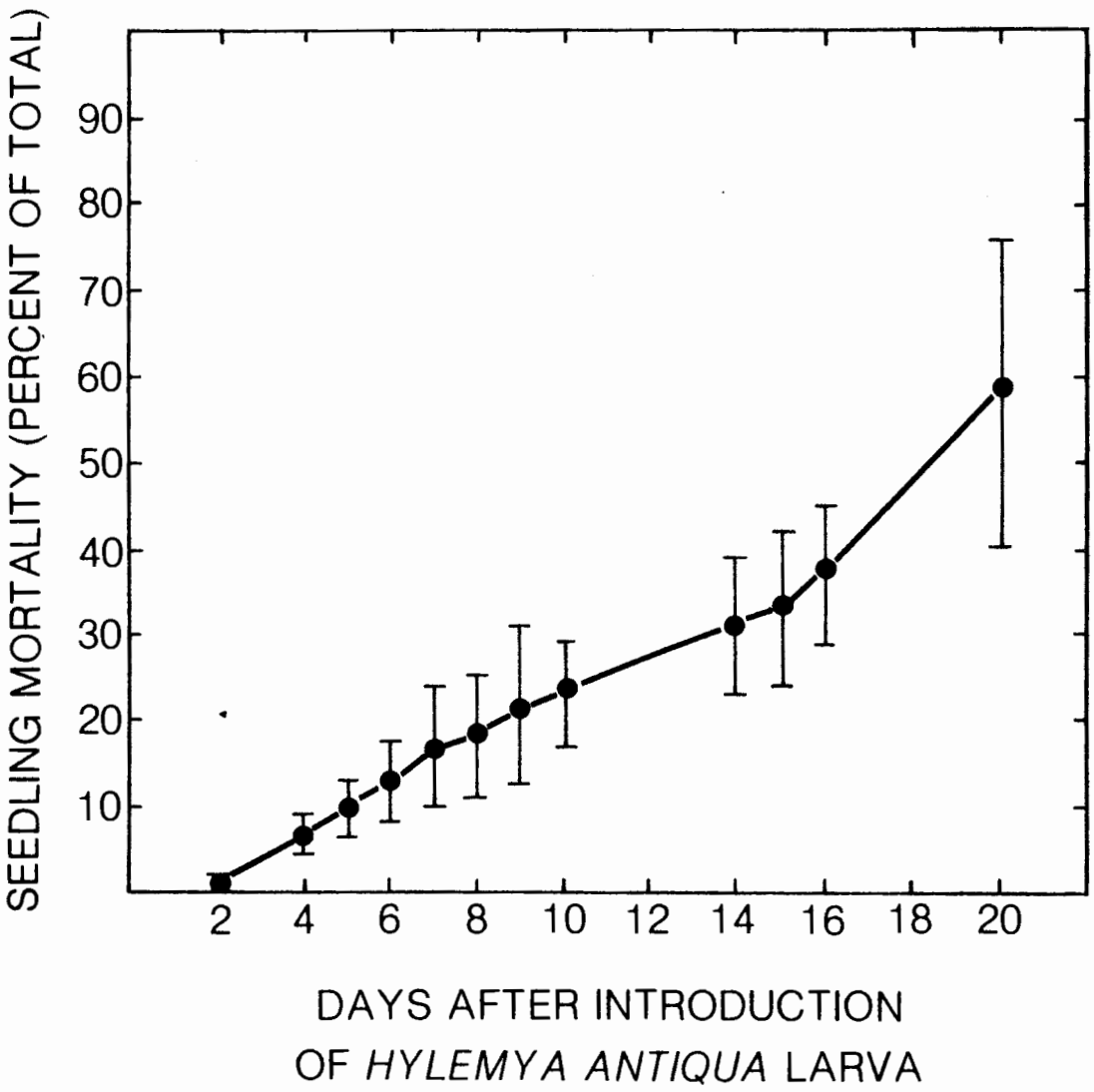
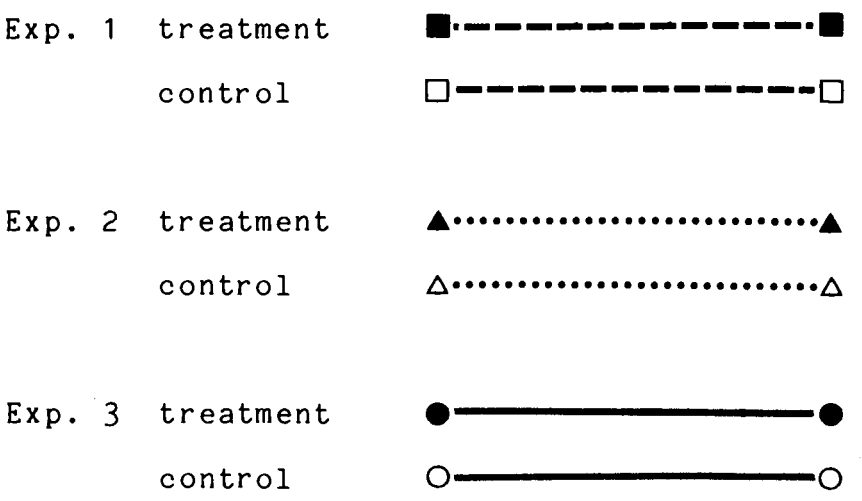
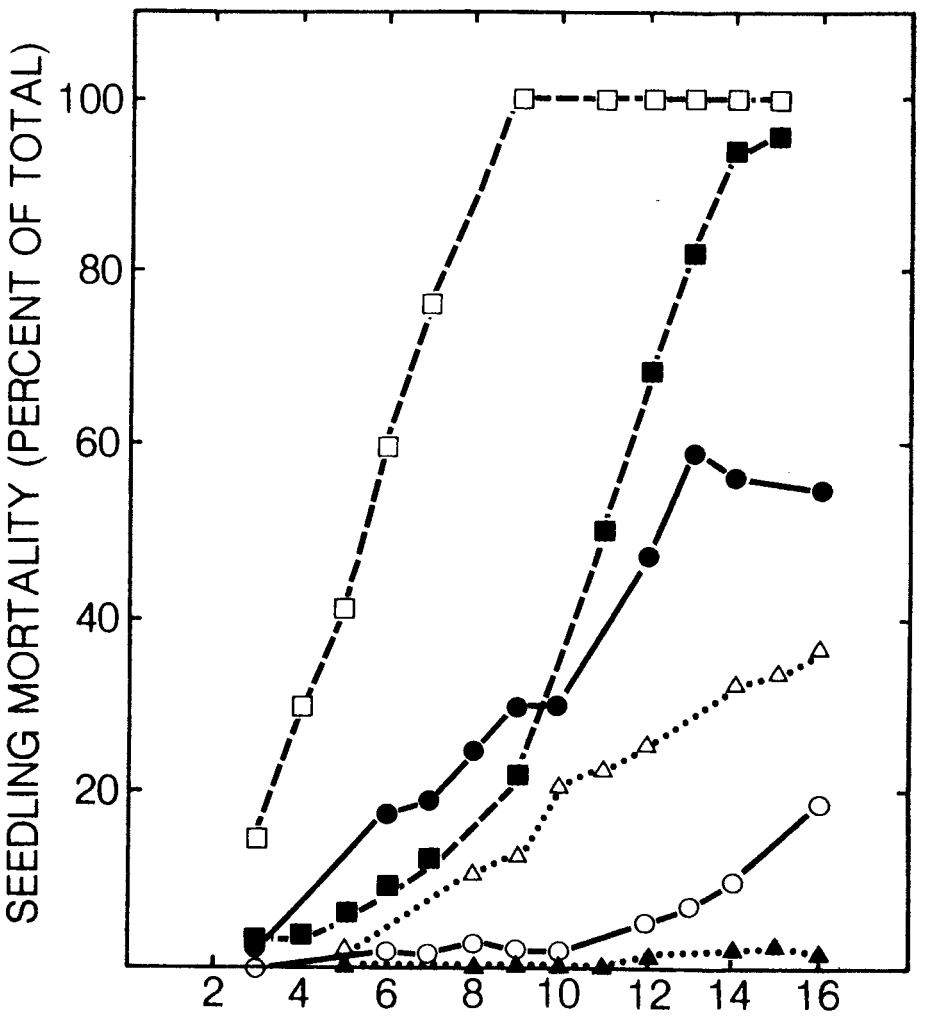

SEEDLING MORTALITY (PERCENT OF TOTAL)
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
DAYS AFTER INTRODUCTION
OF *HYLEMYA ANTIQUA* LARVA

Fig. 2.5 Rate of mortality of onion seedlings due to Hylemya antiqua larval feeding following exposure of pretreated plants to ovipositing flies: Exp. 1, ca. 150 females for 60 min.; Exp. 2, ca. 150 females for 40 min.; Exp. 3, 3 females for 48 h.




SEEDLING MORTALITY (PERCENT OF TOTAL)
100
80
60
40
20
2
4
6
8
10
12
14
16
DAYS AFTER EXPOSURE TO
OVIPOSITING *HYLEMYA ANTIQUA*

DISCUSSION

A. Host Selection Principles

Host plant chemicals acting as attractants, arrestants, stimulants, repellents or deterrents are hypothesized to be the basis of host selection by insects for both food (38, 55, 61, 68, 114, 119) and oviposition (14, 22, 69, 119, 147). Many of these compounds have been classified as "secondary" plant chemicals since they have no known function in basic plant metabolism and their occurrence in plants is sporadic (42, 154). In the case of host selection by H. antiqua, the key stimuli are thought to be volatile sulfur compounds characteristic of onion odour, which act as attractants and oviposition stimulants (90, 91, 147, 148).

Numerous other host-insect interactions appear to be determined by host attractants and stimulants. Mustard oils function as olfactory attractants, arrestants, biting incitants and oviposition stimulants toward pests of cruciferous plants, e.g., cabbage butterflies, Pieris rapae L. and P. brassicae L. (136); diamond back moth, Plutella maculipennis Curt. (48, 136); mustard bettle, Phaedon cochleariae Fab. (135); and cabbage root fly, Erioischia brassicae Bouche (140). Mustard oil glucosides, e.g. sinigrin, on the other hand, are feeding stimulants for cabbage butterflies and the diamond-back moth (33, 136). An oviposition

stimulant (methyl-iso-eugenol) for the carrot rust fly, Psila rosae F. has been isolated from carrot leaves (22).

Although stimulants are prominent in the chemosensory bases of host recognition among insects, Jermy (69) observed that the presence of a single deterrent compound from most non-host plants was sufficient to override the attractants of the natural host for both feeding and oviposition. This observation provides the rationale for the use of the dual-choice bioassay to reveal oviposition deterrents toward H. antiqua. Jermy (68) has suggested that deterrent compounds may be more significant than attractants in determining the host range of phytophagous insects; many plants may have attractants to a particular insect, but only those plants lacking deterrents (or possessing deterrents to which the insect displays tolerance) are suitable host species.

In the light of Jermy's hypothesis, if the onion volatiles stimulating oviposition behaviour of H. antiqua are not unique to the host species, then other plants, but not onions, should contain deterrents. If the stimulants are unique to Allium host species, these compounds alone would be sufficient to explain host selection.

The finding that the oviposition behaviour of H. antiqua is deterred by components of a wide variety of plant extracts provided temporary support for Jermy's hypothesis.

When, however, deterrency was found also in onion (and garlic) extracts, it became clear that neither stimulants nor deterrents isolated from plant tissue are the sole determinants of the host range of H. antiqua.

An alternative hypothesis, taking into account the presence of stimulants and deterrents in host plants, is that a deterrent:stimulant ratio forms the basis of insect selection of host species (37, 92). Host preference by the boll weevil is thought to be determined by the relative proportions of stimulants, attractants, repellents and "oviposition suppression factors" in cotton and other host plants (92). Isothiocyanate stimulated oviposition behaviour in the cabbage butterflies, even in the presence of coumarin, a deterrent in clover, but did not elicit a response when placed on tomato leaves due to "unknown inhibitory substances" in tomatoes (49).

As investigations to identify the chemical stimuli for plant selection and other forms of insect behaviour have progressed, workers (21 38, 50, 62, 63, 64, 65) have recognized that host specificity cannot be entirely explained even in terms of quantitative differences in both deterrent and stimulant compounds. Many different chemicals, both primary (i.e. nutrient) and secondary, are involved (16, 121, 137).

Insect choice of a host plant for food or egg-laying includes several stages of behaviour: host recognition and

orientation, often involving volatile cues, and initiation and maintenance of contact with the host, involving gustatory stimuli (15). Bernays and Chapman (21) reported that for locusts, phagostimulants such as sugars, lipids, amino acids, vitamins and organic acids which activate chemoreceptors upon biting allow host recognition, but continued feeding is dependent on a balance between phagostimulants (primary and secondary) and inhibitors (secondary).

The onion volatiles stimulating oviposition behaviour in H. antiqua are perceived through olfactory receptors on the antennae and maxillae of the fly. My experiments have shown that both BSE and OB extracts deterred the oviposition behaviour of H. antiqua by a contact mechanism. It appears likely that contact receptors represent a 2nd or 3rd order checkpoint, in this case, providing the onion fly with further information on the suitability of an oviposition site. If a broad host range among insects is determined by a combination of tolerance to most deterrents and positive response to most feeding stimulants and cofactors (61, 62, 63, 64, 65, 122), then it is reasonable to predict that mono- and oligophagous insects (i.e. insects with narrow host ranges) should be dependent on a single or few host species through a high sensitivity to deterrents and/or a high specificity for special stimulants. The onion fly appears to fit the latter pattern.

Interactions among active compounds at either the receptor or the central nervous system level may influence the information an insect receives from chemosensory cells. The oligophagous tobacco hornworm, Manduca sexta Johanssen, is sensitive to deterrents present in tomato (120), one of its host plants. The receptors sensitive to the distasteful substances are inhibited, however, in the presence of high sucrose concentrations. It was suggested that the firing frequency of the receptor cell monitoring deterrents was perhaps below a critical threshold, or that the strong stimulation of other receptors compensated for rejection stimuli (35). Adams and Bernays (1) observed that the effects of feeding deterrents to locusts were additive and that both additive and synergistic interactions occurred among phagostimulants to these insects. As is the case with tobacco hornworm, high levels of sucrose override the effects of deterrents to locust feeding.

If oviposition deterrents naturally occur in onion tissue, they may be localized in areas unavailable to contact by H. antiqua or possibly present in precursor form. If oviposition deterrents are naturally present and gravid flies receive stimuli from these compounds, it is likely that interactions with other chemical compounds (e.g. sugars), as illustrated for the locust and tobacco hornworm, moderate the deterrent stimuli. An examination of the effects of BSE on the seed corn maggot (pest of beans) would possibly give more light on the potential role of oviposition deterrents in the host plants of Hylemya species.

The role of chemical stimuli in eliciting insect behaviour is further complicated by the effect of concentration. Dethier (36) stated, "For every attractant so far tested there is a concentration in excess of which the substance acts as a repellent." Adams and Bernays (1) noted that phenolic compounds were increasingly deterrent to locust feeding with concentration. The oviposition deterrent activity of onion oil to Pieris brassicae L. and P. napi L., both larval pests of onions, is thought to be due to a concentration effect (86). In the present study, I observed that concentration influenced the oviposition deterrent activity of BSE. Although volatiles would be removed by the extraction procedure, contact attractants and deterrents may still be present. The 10^4 dilution of BSE could change the relative activities of a mixture of compounds resulting in a change in the proportion of attractant:deterrent stimuli. Interactions of this nature illustrate the complexity of the chemical bases of insect-plant relationships.

B. Field Control with Oviposition Deterrents

Control measures for onion maggot in the field (85) presently consist of:

1. trap crops, i.e. cull onions or deeply planted sets,
2. poisoned sweet bait onions, and
3. chemical treatments, i.e. organochlorines and organophosphates.

Residues with chemical control are not a problem, but the onion maggot has developed considerable resistance to organochlorines and some tolerance to organophosphates (85). Other possibilities for control currently being investigated are:

1. genetic control, i.e. sterile male (insect) releases (138), and
2. chemical and colour attractants plus sticky traps (146).

The latter method is proposed as a screening technique in conjunction with chemical control.

Recently, a number of feeding deterrents have been isolated and characterized as a result of an intensive screening programme at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. Workers (70, 94, 99) are hopeful that some of these antifeedants may be used as protective sprays to control insect pests. It is possible that the same potential for control exists with oviposition deterrents (69). The application of plant extracts containing oviposition deterrents to H. antiqua on field-grown onions for control of onion maggot damage has 2 practical limitations: 1. persistence, and 2. efficiency.

Since BSE oviposition deterrent activity was shown by dual-choice bioassay to persist for up to 4 d, it appears that after 2 d gravid flies tolerated the presence of deterrents in a no-choice situation. This suggests that any application of

oviposition deterrents for onion maggot control should be made in conjunction with baits having attractants (cull onions or chemical stimulants).

Several reports (17, 156) have indicated the large feeding potential of individual onion maggots. Loosjes (85) observed a ratio of 1:11 for seedlings on which egg batches were laid to damaged seedlings in the field. In the present study, a single larva was shown to destroy $\geq$ 24 seedlings (12-d-old) within 19 d. The specific activity of plant extracts containing oviposition deterrents to H. antiqua would therefore have to be increased considerably to control maggot damage to within the economic threshold level of 10 % (85).

A second approach to the use of deterrents (feeding or oviposition) in the control of insects is the breeding of host varieties with higher levels of these chemicals. Manipulation of a range of compounds would provide a more flexible defense strategy for plants and create less selection pressure on insect populations than occurs with the manipulation of a single chemical - whether synthetic compound or natural plant product.

CHAPTER 3

GENERAL DISCUSSION

Plant Resistance to Pathogens and Insect Pests

Plants resist disease and insect attack at several levels of interaction. Volatile chemicals and leachates act at distances to repel potential pests or induce host orientation in pathogens and insects alike. At close range, the defense lines of a plant include both physical (mechanical) and chemical ('secondary' compounds) barriers, some of which may preexist contact with an invader and others of which are induced during the plant-pest interaction. The purpose of this discussion is to compare the chemical bases (in particular, the inducible chemicals) of plant resistance mechanisms to insects and pathogens.

Chemicals involved in the formation of post-infectional barriers fall into 2 main categories (19):

1. wound toxins, and
2. phytoalexins.

Wound toxins are antimicrobial substances formed within minutes or hours of nonspecific cellular injury by the mixing of formerly localized precursors and enzymes. The more common precursor types are polyphenols, glycosides or esters and sulfoxides. Increased levels of peroxidase and polyphenol oxidase enzymes convert polyphenols to the toxic o- and p-quinones (enzyme and metabolic inhibitors). Phenolic

glycosides may also be converted to quinones. Benzoxazinone, cyanohydrin, isothiocyanate and lactone glucosides are enzymatically hydrolyzed to form antimicrobial aglycones (antifungal ED_{50} values 1 to 100 ppm). The antibiotic thiosulfinates are found in the wound sap of garlic (e.g. allicin) and onions [e.g. methyl- and propyl-thiosulfinates; (19)].

Phytoalexins, on the other hand, do not occur to any appreciable extent in healthy tissues and their synthesis is energy-dependent. These antimicrobial (specifically, antifungal) compounds may be induced by a variety of agents: fungi, bacteria, viruses, toxic chemicals, microbial metabolites and adverse physical treatments. The appearance of these compounds is delayed relative to the formation of wound toxins, but phytoalexins accumulate at higher concentrations and are more persistent. Toxicity of these chemicals to microorganisms is usually less (ED_{50} 25 to 1000 ppm) than that of wound toxins. Phytoalexins may be acetate-derived (e.g. ipomeamarone, gossypol, rishitin, phytuberin, steroid alkaloids) or hydroxycinnamate-derived (e.g. cinnamic acid and other free acid derivatives, coumarins, lignins, and esters).

The majority of plant chemicals acting as insect deterrents fall into the preformed or passively formed group of compounds, rather than the inducible phytoalexin-type compounds.

Saponins (glycosides of steroids) from legumes have been extensively studied for their growth-inhibiting activity, i.e. 'antibiosis', toward insects (6 7, 8, 43, 123, 124). When fungi were exposed to the saponin cyclamin at 5 to 200 ppm, all species tested were killed (118). Sapogenin aglycones, e.g., medicagenic acid, also have considerable antifungal activity (84).

Juglone is the quinone product of a phenolic glycoside. This compound is a feeding deterrent to bark beetles (44) and also possesses antifungal activity. In fact, Fawcett and Spencer (41) showed a high potential for juglone as a protective fungicide. Various antimicrobial phenolics, e.g., p-hydroxybenzoic, caffeic, ferulic, protocatechuic, vanillic and p-coumaric acids, possessed little deterrent activity singly, but as a mixture were significantly deterrent through an additive effect (1). The polyphagous green peach aphid tolerated tomatine and solanine deterrents but did not feed in the presence of phlorizin, salicin and the alkaloids, quinone and berberine (122).

Cyanogenic glycosides afford plant resistance to insects by nonpreference (feeding deterrency) and antibiosis mechanisms (71). Cyanohydrins are fungitoxic compounds formed upon hydrolysis of cyanide-containing glycosides (19).

Benzoxazinone derivatives, characteristic of the Family Graminaceae, are wound toxins implicated in plant resistance to fungi and insects. Reports conflict as to the antifungal activity of the precursor benzoxazinone glucosides (25, 149). Benzoxazinone (DIMBOA) is toxic to bacteria and has antifungal activity with ED_{50} values of 1 to 20 ppm (25). In addition, DIMBOA conditions resistance in corn to the European corn borer (Ostrinia nubilalis Hubner) by a nonpreference mechanism (112). The aglycone is somewhat unstable and yields benzoxazolinone (MBOA) a compound having less biological activity, i.e. ED_{50} 50 to 500 ppm (19) and no insect deterrent activity (77).

Gossypol and related terpenoid compounds in cotton plants provide resistance against a variety of organisms. The mechanisms of resistance against insect larvae (Heliothis zea Boddie, H. virescens F., and Spodoptera littoralis Boisduval) include both deterrency and antibiosis [inhibition of proteolysis; (95, 96, 97)]. Although gossypol occurs in the glands of healthy cotton plants, it is considered a phytoalexin because it has antifungal activity and it accumulates at higher levels in resistant varieties the first few days after inoculation, than in susceptible tissue (18). A dihydroisocoumarin with antifungal properties has been isolated from carrots (133), while the presence of coumarin in sweet clover leaves deters the feeding of blister beetles (46) and vegetable weevils (88).

In some cases, it appears that insect pests have specifically adapted to toxic compounds as chemical cues for host orientation, e.g., isothiocyanates (mustard oils) are attractants and stimulants for pests of the Brassicae. Alkaloid glycosides deter many pests (55), but are phagostimulants for the Colorado potato beetle, Leptinotarsa decemlineata Say (105). Such specific adaptation by insects is associated with mono- or oligophagous feeding habits.

It is not surprising that wound toxins should be more involved in plant resistance to insects than are phytoalexins. Inducible (delayed) resistance mechanisms are less likely to be effective against mobile pests having a relatively short contact time with host plants, than against pathogens which establish a chronic relationship with their hosts.

Most plant resistance mechanisms against insects are chemically undefined (76). Biological activity for many individual plant chemicals has been established, but as with research on phytoalexins, a clear demonstration of the involvement of such compounds at appropriate sites in vivo is lacking. An understanding of the chemical bases of host resistance in plant-insect and plant-pathogen interactions will improve the target selectivity and predictability of control and management practises which utilize plant natural products.

Summary

The present study has contributed to the applied sciences in several ways:

1. development of a bioassay which may be used in further screening studies of plant natural products for potential as fungicides,
2. discovery of oviposition deterrents to H. antiqua occurring commonly in plant tissue extracts including the specific host plant of this insect, i.e. onion, and
3. demonstration in the laboratory of partial control of onion maggot damage to soil-grown onion seedlings by using plant extracts which contain oviposition deterrents to gravid H. antiqua as protective sprays.

These results provide 2 immediate possibilities for further research:

1. the use of the 'inoculum plus fungitoxicant' bioassay to test other phytoalexins and various natural and/or synthetic chemicals as fungicides, and
2. the isolation and characterization of oviposition deterrents to H. antiqua in plant tissue extracts.

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