THE COMMERCIAL IMPLEMENTATION OF

AND

EVALUATION OF ENVIRONMENTAL EFFECTS ON <u>AMBLYSEIUS</u> <u>CUCUMERIS</u> OUDEMANS AS A BIOLOGICAL CONTROL AGENT OF <u>FRANKLINIELLA</u> <u>OCCIDENTALIS</u> PERGANDE

by

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Title of Thesis/Project/Extended Essay

The commercial implementation of and evaluation of environmental

effects on Amblyseius cucumeris oudemans as a biological control

agent of Frankliniella occidentalis pergande

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ABSTRACT

The western flower thrips (WFT), Frankliniella occidentalis Pergande, has recently become a major pest of greenhouse vegetable crops. In cucumbers, the potential for leaf and fruit damage necessitates the use of broad-spectrum insecticide applications, upsetting biological control systems. Thus a biological control agent was sought for WFT. The phytoseiid predator, <u>Amblyseius cucumeris</u> Oudemans, had a broad host range with a preference for thrips, an observed numerical response to thrips populations, was abundant and competitive in nature, and was a promising biological control agent for WFT in a preliminary laboratory assessment. I implemented a 3-farm trial to determine its effectiveness in commercial conditions.

On 2 farms, WFT numbers remained low until increasing rapidly in Autumn. On the third farm, WFT numbers increased slowly at first and then rapidly after pesticide use started in mid-June. Paired-leaf studies indicated the suitability of <u>A</u>. <u>cucumeris</u> for commercial biological control of WFT. I then tested the effects of daylength and relative humidity (RH) on the performance of <u>A</u>. <u>cucumeris</u> in the laboratory. Daylength had little influence on egg hatch, developmental rate of juveniles, or longevity and oviposition of adults. An RH threshold of 70% was found, below which egg hatch, developmental rate of juveniles, and longevity and oviposition of adults was retarded. Although periodic episodes of low RH may not effect <u>A</u>. <u>cucumeris</u> as much as continuous exposure, this RH sensitivity may explain in part the rapid Autumn increase of WFT in 2 greenhouses.

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I would like to thank Dr. Robert Costello for first stimulating my interest in greenhouse pest management; Don Elliot and Dr. Linda Gilkeson for continued consultation and encouragement throughout my research; Dr. J. E. Rahe for lending the use of his mind; Dr. D. R. Gillespie for making his time, resources, knowledge, and ideas freely available; Dr. John H. Borden for his constant stimulation, encouragement, criticism, and guidance; and finally my family for tolerating the crazy life of a part-time student.

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CHAPTER 1: INTRODUCTION

A. GREENHOUSE INDUSTRY

The greenhouse industry in British Columbia encompasses the culture of flowers, tropicals, ornamentals, and vegetables. The main vegetables are tomatoes, peppers, and long English cucumbers; they account for some 43% of the total industry by area. Gross farm gate receipts for this sector were ca. \$23 million in 1988 (B. Mauza,¹ pers. comm.).

Cucumbers and tomatoes have been grown in B.C. greenhouse culture since the late 1960's; peppers are a more recent introduction, starting in the mid-1980's. Early crops were grown in the soil with periodic applications of manure or synthetic fertilizers. To avoid disease and nutrient problems, hydroponic culture in a soil free substrate was introduced. Hand or mechanical watering devices were replaced by metered drip irrigation of nutrient solution, and the substrate shifted from peat through peat-perlite to sawdust and some rock wool culture. During this evolution, the greenhouses became brighter (fewer supporting structures for less shade) with improved climatic

Provincial Greenhouse Specialist, B.C. Ministry of Agriculture and Fisheries, Abbotsford, B. C.

controls. These trends culminated in computer controlled atmosphere and nutrient feeding systems.

B. CUCUMBER & PEPPER CULTURE

The growth requirements for cucumber and pepper are similar. Both need light, CO_2 , water, and nutrients. During summer, when light is abundant, CO_2 is usually the limiting factor, so compressed CO_2 is introduced to accelerate growth. Acceptable temperatures range from 18 to 28°C, with optima from 21 to 24°C (Anonymous 1986).

Pruning and training can improve productivity by limiting unnecessary foliage and maximizing light and space utilization (Anonymous 1986). Peppers are started in November. Three stems per plant are trained up separate strings. Side shoots are trimmed to the first or second bunch of flowers. Plants reach a maximum height of 2 to 3 m by the following October, when the crop is removed. Cucumbers are usually seeded in December or January and transplanted to the greenhouse one month later. One main stem per plant is trained up a string; it reaches a horizontal supporting wire at 2 to 2.5 m in about 30 days. Two laterals are trained about 1 m from the main stem along the wire. From them, side-shoots grow downwards, bear cucumbers, and are removed to leave room for future side-shoots. About 6 sideshoots per plant are maintained at any given time. In October the crop is removed.

C. INTEGRATED PEST MANAGEMENT

Since a white plastic ground cover is used in hydroponic culture, weeds are only a minor problem. Occasionally, weeds will appear in splits in the ground cover or around the borders of the greenhouse, where they may harbour populations of the two-spotted spider mite, <u>Tetranychus urticae</u> Koch, as well as whiteflies, thrips, and some diseases (personal observation).

Diseases are mostly controlled by keeping temperature, relative humidity, light, and CO₂ favourable for plant growth and unfavourable for disease development. Nutritional manipulation, such as increased calcium or silicone, may also affect disease incidence (Adatia and Besford 1986; J. Menzies,¹ pers. comm.). Resistant cultivars are used increasingly for control of such diseases as powdery mildew in cucumbers. Intractable disease problems are suppressed with chemicals. For example, botrytis stem rot may be spot-treated with benomyl. However, these chemical applications often have a detrimental effect on biological control of arthropods (D. Elliot,² pers. comm.)

Arthropod pest management has historically consisted of applications of broad-spectrum pesticides against <u>T</u>. <u>urticae</u>, the onion thrips, <u>Thrips tabaci</u> Lind., the greenhouse whitefly <u>Trialeurodes vaporariorum</u> Westwood, and various aphids. Resistance to pesticides led to the local adoption, in the late

² President, Applied Bio-Nomics Ltd., Sidney, B.C.

¹ Agriculture Canada Research Station, Agassiz, B. C.

1970's, of biological control programs against <u>T</u>. <u>urticae</u> and <u>T</u>. <u>vaporariorum</u>, using <u>Phytoseiulus persimilis</u> Athias-Henriot (Tonks and Everson 1977) and <u>Encarsia formosa</u> Gahan (Gould 1971), respectively. As these programs were refined and improved, they have been adopted by most cucumber and pepper growers, resulting in reduced dependance on pesticides and improved pest control (Costello et al. 1984). By 1984, biological controls were available for every major insect pest in greenhouse vegetable production (Hussey 1985a)

Before plastic floor coverings became standard in the mid-1980's, pseudopupae of <u>T</u>. <u>tabaci</u> dropped to moist soil. There, infection by fungal diseases, such as <u>Entomophthora parvispora</u> and <u>E</u>. <u>thripidum</u>, kept populations low (Hussey 1985b). Occasional outbreaks were easily controlled with ground sprays of Diazinon or Thiodan (endosulfan) (R. Costello,³ pers. comm.). However, the use plastic floor coverings offered a dry pupation site unfavourable to the development of pupal diseases.

At the same time that these coverings were adopted, the western flower thrips (WFT), <u>Frankliniella occidentalis</u> Pergande, appeared. <u>F. occidentalis</u> has a similar life cycle to <u>T. tabaci</u>. Adults and nymphs feed on the leaves, causing necrotic areas; in the flowers; and on developing fruit, causing curling and surface striations. Adult females insert eggs in leaf or stem tissue; these hatch in 4-6 days. After 2 nymphal instars (9-12 days), pseudopupae appear and drop to the ground.

³ Entomologist, B. C. Ministry of Agriculture and Fisheries, Cloverdale, B.C.

Adults emerge 7-10 days later, live for up to 40 days and lay 1 to 2 eggs per day (Bryan and Smith 1956).

While <u>T</u>. <u>tabaci</u> is easily controlled with ground sprays, WFT requires foliar sprays of broad-spectrum, non-integratable insecticides which often destroy the integrated biological control programs for other pests. Therefore, biological control of WFT was desired.

<u>Amblyseius cucumeris</u> Oudemans, an uncommon predator in Canada (Anderson et al. 1958) was found to be a predator of nymphal WFT in the Netherlands (Ramakers 1978). It was selected by DeKlerk and Ramakers (1986) as the primary candidate for biological control of WFT, since it appeared to meet the requirements of a good control agent as outlined by ZwÖlfer et al. (1976): a fairly broad host range, preference for thrips, low variation in the ratio of predator to prey numbers, abundance in its natural habitat, and competitive ability.

The life cycle of <u>A</u>. <u>cucumeris</u> is ca. half as long as that of WFT (Gillespie and Ramey 1988). Eggs hatch to non-feeding larvae, which moult to nymphs after 1 day. The protonymphs and deutonymphs develop in 5-7 days, then moult to adults. After a 2-day, pre-ovipositional period, 1 to 5 eggs/day are laid for up to 40 days (El-Badry and Zaher 1961; Chant and Hansell 1971).

Preliminary laboratory studies (D. R. Gillespie,⁴ unpublished data) concerning longevity, fecundity, and prey preference indicated that <u>A</u>. <u>cucumeris</u> would be an effective

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Agriculture Canada Research Station, Agassiz, B. C. Canada

biological control agent of WFT. Although it feeds and develops on several acarine pests (Muma 1971; Jeppson et al. 1975), it seems to prefer thrips nymphs, consuming up to 6/day (DeKlerk and Ramakers, 1986). In a small-scale greenhouse trial, the predator successfully controlled a <u>T</u>. <u>tabaci</u> population on cucumbers (Gillespie 1988). Since WFT and <u>T</u>. <u>tabaci</u> are taken equally well in the laboratory, it was predicted that <u>A</u>. <u>cucumeris</u> would control WFT in commercial greenhouses. <u>A</u>. <u>cucumeris</u> is easily mass reared, due to its polyphagous nature. Schliesske (1981) reared <u>A</u>. <u>cucumeris</u> on grain mites in a bran substrate. This technique has been refined to produce large quantities of <u>A</u>. <u>cucumeris</u> on a commercial basis by Koppert B.V. in the Netherlands and Applied Bio-Nomics Ltd. of Sidney, B.C

D. OBJECTIVES

My principal objective was to determine the effectiveness of <u>A</u>. <u>cucumeris</u> as a predator against WFT in a commercial cucumber production setting. Because there were variable results in commercial greenhouses further work was undertaken in the laboratory. This augmented concurrent inquiries by other researchers on environmental factors affecting the success of <u>A</u>. <u>cucumeris</u>.

CHAPTER 2: COMMERCIAL GREENHOUSE STUDIES

A. INTRODUCTION

The importation and use of natural predators requires several steps (DeBach et al 1976; Anonymous 1979). Collections from the natural ecosystem must first be made. Analysis of quantitative population data will offer an indication of the value of a candidate. Laboratory studies then provide data on the life history and other aspects of the predator. These data may confirm its potential usefulness and refine the details of its use. But the acid test of usefulness as a biological control agent is field trials under commercial conditions.

The studies described in this chapter were preceded by only minimal laboratory studies. Thus, one could not know whether <u>A</u>. <u>cucumeris</u> was being used to its best advantage. Inherent with such trials is a high probability of type-II error, rejecting <u>A</u>. <u>cucumeris</u> as a biological control agent when it should be accepted. However, rapid commercial development of a biological control agent can occur, especially if resources are limited. As described previously, the greenhouse cucumber industry urgently needed an alternative method of managing WFT. Since the predator had already been tested on related prey, and a commercial rearing system was available, rapid development was used in this situation.

Another inherent disadvantage to commercial-setting studies is their dependance on commercial constraints. The farmer may delay the distribution of a predator throughout his greenhouse.

He takes a personal risk in such a project, and may choose to abort the project and use alternative management techniques at any time. Thus the continuity of the project is subject to the farmer's perception of its success.

The availability of predators is also subject to the limits of commercial production. Malfunctions in the production system occasionally resulted in insufficient supplies for the participating growers.

The final problem with commercial-setting trials is the lack of control between farms. Each participant in this study had slightly different humidity, CO₂, and light relations, fertilizer regimes, feeding schedules, and pruning and harvesting methods. As well, the overall pest complex (including diseases) and the growers' reactions to it varied from farm to farm. Although some researchers feel such variability is beneficial by providing a wider range of test conditions, it is detrimental to controlled experimentation.

Despite the drawbacks to early commercial trials, they were considered warranted in this situation, with respect to rapid development and limited resources. The objectives of this study were to determine the commercial acceptability of <u>A. cucumeris</u> as a biological control agent against <u>F. occidentalis</u>.

B. MATERIALS AND METHODS

i. A. cucumeris Cultures

<u>A</u>. <u>cucumeris</u> were cultured commercially on bran by Applied Bio-Nomics Ltd.⁵ using the method of Ramakers and van Lieburg (1982). Shipments were made in cooled styrofoam packages. <u>A</u>. <u>cucumeris</u> in bran were packed in paper bags at ca. 0.5 L of bran mixture per bag. Delivery to the growers was made the day following packaging.

ii. Greenhouse activities

Three greenhouse cucumber growers in the Lower Fraser Valley, British Columbia, participated. Farms A, B, and C had 0.4, 0.4, and 0.8 ha in cucumbers, respectively. All growing activities were carried out by the grower or his employees. Pest control decisions, including whether to use chemicals, were also made and carried out by the grower. <u>A</u>. <u>cucumeris</u> were provided free of charge; it was each grower's responsibility to distribute them throughout the greenhouse. Since each grower was interested in the success of the biological control project, <u>A</u>. <u>cucumeris</u> were relied upon for as long as possible.

The bran containing <u>A</u>. <u>cucumeris</u> was sprinkled on 1 mature leaf per 1-2 plants throughout the greenhouse at about 100 predators/plant. The first release on plants was made immediately after transplanting on Farms A and B, and 2 weeks

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Box 2637, Sidney, B.C. V8L 4C1 Canada

before transplanting on Farm C. Releases were made thereafter at about biweekly intervals.

iii. Population Monitoring

Monitoring for WFT, A. cucumeris, and the T. urticae commenced on March 7, and continued at weekly intervals until crop removal. WFT adults were monitored with yellow-plastic sticky traps (4 x 6 cm) (Olson Products⁶) hung near the top of the canopy. Trap counts were used to identify areas of high thrips infestation within each greenhouse. WFT nymphs, A. cucumeris, and <u>T</u>. <u>urticae</u> were monitored with a leaf-washing techniue. Four to 6 samples of 5 to 8 mature leaves each were collected weekly from areas of high thrips infestation within each greehouse. All leaves in each sample were washed with a jet of water, dislodging larval thrips and predators. The water was passed through 10-, 32-, and finally 100-mesh screens. The 10- and 32-mesh screens excluded bits of leaf and trash, the 100-mesh screen caught thrips larvae, predators, and some predator eggs. Arthropods from the 100-mesh screen were washed into a petri plate and counted under a binocular microscope.

Overall monitoring results as well as results within areas of high thrips infestation on each farm were recorded. Data collected were: the numbers of WFT and <u>A</u>. <u>cucumeris</u>; levels of <u>T</u>. <u>urticae</u> on an ordinal scale (0= 0 <u>T</u>. <u>urticae</u>/leaf, 1= 0.1-0.4/leaf, 2= 0.5-2.9/leaf, 3= 3-6.9/leaf, 4= 7-29/leaf, 5≥ 30/leaf); pesticides used; and the date and quantity of \underline{A} . <u>cucumeris</u> releases.

iv. Characteristics of A. cucumeris Behaviour

Five paired-leaf experiments were conducted to assess four characteristics of <u>A</u>. <u>cucumeris</u> predation. In each experiment, leaf pairs were selected from 1 plant or 2 adjacent plants but never from the same shoot within a plant, except in the experiment to determine relative speeds of shoot colonization. Each leaf was similar to the other in all ways but the factor being explored. Leaves on which <u>A</u>. <u>cucumeris</u> were released were not included. Five to 8 pairs were pooled to make one replicate; each experiment had 11 to 26 replicates. Arthropod counts were taken using the wash/screen technique described above.

Plant height preference was determined by selecting leaf pairs of similar size, age, and <u>T</u>. <u>urticae</u> infestation as determined by visual inspection. One member was selected from the top of the canopy at a height of >2m, the other at a height of ca. 1.2 m.

Leaf age preference was determined by selecting leaf pairs of similar height (ca. 1.5 m) and <u>T</u>. <u>urticae</u> infestation. One leaf was 2 to 4 months old, brittle, and >30 cm in diameter, the other was ca. 4 weeks old, supple, and ca. 20 cm in diameter.

Interaction with \underline{T} . <u>urticae</u> was determined by selecting leaf pairs of similar age, size, and plant height. One leaf was infested with \underline{T} . <u>urticae</u>, the other had no colonies. The relative speeds of shoot colonization by <u>A</u>. <u>cucumeris</u> (released on mature leaves) and WFT are important in the success of biological control. Speeds of colonization were determined with 2 experiments:

 Leaf pairs of similar <u>T</u>. <u>urticae</u> infestation were selected from the same shoot. One member was a mature leaf (>20 cm diameter), ca. 6 leaves back from the growing tip; the other was the third leaf back from the tip, 8 to 12 cm in diameter.
 Similar to 1. except that the second member was the second leaf back from the growing tip, 5 to 8 cm diameter.

All data were analyzed with Wilcoxon's paired-sample test (Zar 1984). Since the differences between pair members were highly skewed, the paired-sample t test could not be used. The non-parametric analogue is Wilcoxon's paired-sample test.

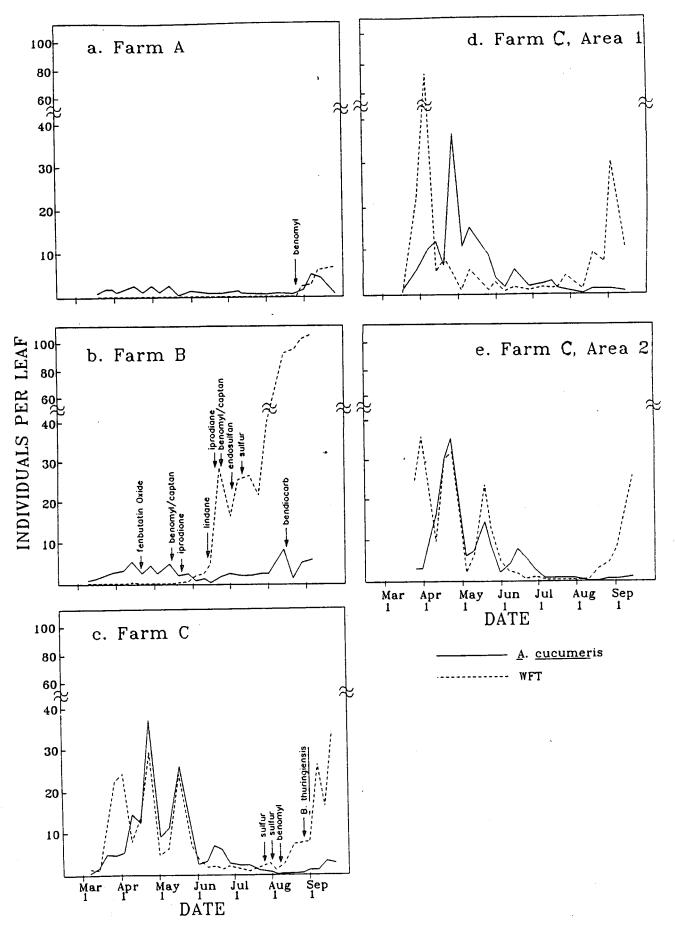
C. RESULTS AND DISCUSSION

i. Population Monitoring

Farm A had very few <u>F</u>. <u>occidentalis</u> or <u>A</u>. <u>cucumeris</u> until September, when the numbers of WFT increased (Fig. 1a). WFT adults were detected at low levels from mid-April through the summer on sticky traps; nymphs were detected on leaves but at levels too low to show on Fig. 1a. Both WFT and <u>A</u>. <u>cucumeris</u> on Farm B remained at low levels until Mid-June, when WFT started increasing, seemingly without hindrance (Fig. 1b). The grower at this farm resorted to foliar treatments of undisclosed

Figure 1. Mean numbers of <u>A</u>. <u>cucumeris</u> and <u>F</u>. <u>occidentalis</u> (WFT) sampled in 3 farms (Figs. 1a- 1c). Figs 1d and 1e depict trends in 2 seperate areas of Farm C (Fig. 1a).

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insecticides in late August and September, so monitoring stopped and biological control attempts with <u>A</u>. <u>cucumeris</u> were aborted.

The population fluctuations on Farm C (Figure 1c) indicated a classic predator/prey response (Huffaker 1976). Numbers of the prey increased, followed after 3 weeks by numbers of the predator. The predator eventually became more numerous than the prey, whereupon both populations collapsed and stabilized at a low level. This trend is more apparent in Figures 1d and 1e, which provide figures for 2 separate areas of Farm C, each slightly out of phase with the other.

The most likely reason for success on Farms A and C, and for failure on Farm B, was pesticide use. <u>A</u>. <u>cucumeris</u> has a very high acute susceptibility to most insecticides and fungicides (D. Elliot,³ pers. comm.), and is tolerant only to fenbutatin oxide. Sub-lethal effects for <u>A</u>. <u>cucumeris</u> are not known, but can affect longevity, fecundity, and development in other arthropods (Irving and Wyatt 1973; Croft and Brown 1975). Oviposition in other <u>Amblyseius</u> spp. is reduced by sub-lethal contact with residual acaricides and fungicides (Nakashima and Croft 1974; El-Banhawy 1976). Thus it is probable that pesticides applied at sub-lethal levels will also hinder the ability of <u>A</u>. <u>cucumeris</u> to suppress WFT populations.

On Farms A and B, <u>A</u>. <u>cucumeris</u> were first introduced before WFT were detected. There was a corresponding season-long suppression of WFT on Farm A. Suppression of WFT on Farm B continued until mid-June, when the first pesticides were used (Fig. 1b). Pesticide use continued, and the WFT population increased without concurrent buildup of the <u>A</u>. <u>cucumeris</u> population, presumably due to a greater impact of the pesticides on <u>A</u>. <u>cucumeris</u> than WFT.

On Farm C, a WFT infestation, overwintered from the previous year, was established on the plants before <u>A</u>. <u>cucumeris</u> were first released. The WFT population grew rapidly until overcome by <u>A</u>. <u>cucumeris</u>; biological control persisted until the end of summer.

Farms A and C, in which biological control was demonstrated, both experienced a late-season breakdown of control. This breakdown may be due to 2 factors. Possibly <u>A</u>. <u>cucumeris</u> may have entered daylength-induced diapause early in the fall, releasing WFT. Although <u>A</u>. <u>cucumeris</u> have been observed in the greenhouse under short-daylength conditions, they are lethargic and adhere tightly to the leaf veins.

Secondly, cooler ambient (outside) air temperatures may have resulted in lower relative humidity (RH) in the heated greenhouse air. I suspect that lower RH may have adversely affected the performance of <u>A</u>. <u>cucumeris</u>. Finally, both farms embarked on a rigorous fungicide program in late August. The ability of <u>A</u>. <u>cucumeris</u> to control WFT may have been inhibited by these pesticide applications.

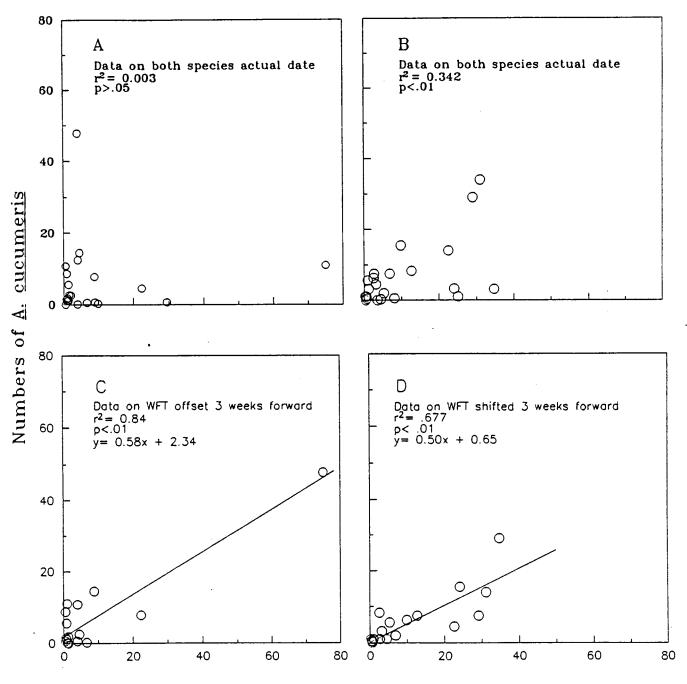
ii. Numerical Response of A. cucumeris to F. occidentalis

In 2 separate areas of high WFT infestation on Farm C, numbers of <u>A</u>. <u>cucumeris</u> were not strongly related to numbers of WFT larvae (Fig. 2a, b). However, if the 3-week lag in A.

Figure 2. Relationship between <u>A</u>. <u>cucumeris</u> and WFT numbers in 2 areas of Farm C.







Numbers of WFT larvae

<u>cucumeris</u> response to WFT is considered (Fig. 1d, e), WFT numbers can be shifted forward by 3 weeks. The last 3 data points can also be dropped to avoid the pesticide-induced response in September. With both adjustments made, <u>A. cucumeris</u> and WFT populations became strongly related (Fig. 2c, d). Thus there appears to be a strong numerical response of <u>A. cucumeris</u> to <u>F. occidentalis</u> populations, with a 3-week lag period.

iii. Characteristics of A. cucumeris Behaviour

No significant preference of <u>F</u>. <u>occidentalis</u> for upper over lower leaves was noted (Table 1, exp. 1). However, if samples had been taken when WFT counts were higher, the population may have been more segregated, as was suggested by visual observations. <u>A</u>. <u>cucumeris</u> levels were also equal on upper and lower leaves. In view of the numerical response between the predator and the prey (Fig. 2c, d), this distribution may reflect that of WFT.

Both <u>F. occidentalis</u> and <u>A. cucumeris</u> showed a highly significant preference for old leaves, with almost 4 times as many WFT and 3 times as many <u>A. cucumeris</u> on old mature as on young mature leaves (Table 1, exp. 2). However, old leaves were ca. twice as large as young leaves (mature leaf size diminishes as the season progresses). When the data for old leaves are divided by 2, the apparent preferences for older leaves become non-significant (Table 1, exp. 3). Accounting for surface area is valid, since the young leaves had been mature in size for at least 1 mo, and WFT nymphs drop off as pseudopupae after 9-12

days, so that observed nymph populations are not residual from original infestations on a younger leaf. Therefore, both WFT and <u>A</u>. <u>cucumeris</u> are distributed equally on leaves of either age.

Significantly fewer WFT were found on <u>T</u>. <u>urticae</u>-infested than on <u>T</u>. <u>urticae</u>-free leaves (Table 1, exp. 4), suggesting aggressive displacement or avoidance behaviour in WFT oviposition. However, significantly more <u>A</u>. <u>cucumeris</u> were found on <u>T</u>. <u>urticae</u>-infested than on uninfested leaves. Apparently, in scarce WFT populations, <u>A</u>. <u>cucumeris</u> is accumulating in <u>T</u>. <u>urticae</u> colonies. Although <u>A</u>. <u>cucumeris</u> probably prefers thrips as prey (D. R. Gillespie, unpublished data), it is a generalist predator (Muma 1971) and appears to utilize <u>T</u>. <u>urticae</u> as an alternate host during shortages of WFT.

A similar mechanism exists in <u>Amblyseius aurescens</u> Athias-Henriot, which will switch from its preferred prey, the strawberry cyclamen mite, to other hosts and plant exudates in the absence of the cyclamen mite, <u>Tarsonemus pallidus</u> Banks (Huffaker and Kennet 1956). Although its development halts, it can survive in the absence of its preferred prey.

Presumably, when WFT populations climb, <u>A</u>. <u>cucumeris</u> will cease relying on <u>T</u>. <u>urticae</u> for survival and switch back to <u>F</u>. <u>occidentalis</u>. This hypothesis is supported by the week relationship ($r^2=0.02$) between <u>A</u>. <u>cucumeris</u> and <u>T</u>. <u>urticae</u> on Farm C, which had high WFT levels and a stronger relationship ($r^2=0.41$) between <u>A</u>. <u>cucumeris</u> and WFT.

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Exp. Number	ractor Studied	reat Treatment	N	Mean	‡SE	α a	Mean	± SE	aa
1	Plant Height	Upper Lower	11	12.30 0.95	±8.10 ±0.32	0.800	2.53 2.15	±0.45 ±0.39	0.415
2	Leaf Age	01d Young	14 14	4.31 1.06	± 1 .80 ±0.44	0 * 000	9.80 3.36	±2.90 ±0.86	0.004
e	Leaf Age, size adjusted	01d Young	14 14	2.16 1.06	±0.92 ±0.44	0.208	4.88 3.36	±1.40 ±0.86	0.778
4	T. urticae	Infested Uninfested	18 18	0.04 0.23	±0.03 ±0.17	0.000	2.52 1.52	±0.44 ±0.19	0.035
Ŋ	Shoot Movement i)	Full-size New	26	3.98 1.38	±1. 20 ±0.40	0.008	6.50 0.81	±1.60 ±0.24	0.000
Q	Shoot Movement ii)	Full-Size Very new	13 13	3.60 0.78	±1.10 ±0.47	0.076	3.07 0.13	±0.40 ±0.03	0.002
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^aWilcoxon's paired-sample test probability level for difference between means for each experiment.

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There were 3 times as many <u>F</u>. <u>occidentalis</u> nymphs on fullsized as on the third youngest leaves, but 8 times as many <u>A</u>. <u>cucumeris</u> (Table 1, exp. 5). The full-sized leaves had 5 times as many WFT as the second smallest leaves, but this difference was not significant (P=0.076). However, there were 24 times as many <u>A</u>. <u>cucumeris</u> on the full-sized as on the second smallest leaves (Table 1, exp. 5).

The low difference in WFT populations between full-sized and third youngest leaves, and the non-significant difference between full-sized and second youngest leaves, indicate that WFT readily attack the youngest growing tips on the plant. However, the 8 and 24 fold differences in <u>A</u>. <u>cucumeris</u> numbers on full-sized verses third and second youngest leaves, respectively, indicate that <u>A</u>. <u>cucumeris</u> moves slowly down a growing shoot and is almost absent from the tip. <u>A</u>. <u>cucumeris</u> may not move to the tip when an abundance of prey is available.

D. EVALUATION OF <u>A</u>. <u>CUCUMERIS</u> AS A BIOLOGICAL CONTROL AGENT OF F. <u>OCCIDENTALIS</u>

Rosen and Huffaker (1983) describe 4 main desirable attributes of a biological control agent: searching capacity, host specificity, power of increase, and fitness and adaptability.

The searching capacity of <u>A</u>. <u>cucumeris</u> is related to prey location and habitat selection. Although prey location in <u>A</u>. <u>cucumeris</u> is unexamined, work with other phytoseiids has demonstrated an orientation to host residues (Hoy and Smilanick 1981). It is probable that a similar non-random prey location mechanism exists in <u>A</u>. <u>cucumeris</u>, perhaps in response to WFT feces.

A. <u>cucumeris</u> appears to disperse uniformly throughout the cucumber canopy. Thus it is able to take immediate advantage of an invading WFT population. However, <u>A</u>. <u>cucumeris</u> are scarce on shoot tips, leaving a window of escape for WFT, which precludes eradication but helps prevent predator starvation. If very high WFT populations exist, <u>A</u>. <u>cucumeris</u> may have to be released on growing shoot tips as well as the rest of the plant to effect quicker biological control.

Another factor reducing starvation of A. cucumeris populations is its broad range of acceptable hosts. Unfortunately, A. cucumeris is life-stage specific for its preferred hosts. Since WFT adults are large and aggressive, and since they embed their eggs in plant tissue, these stages are unavailable as prey. Second-instar nymphs are able to defend themselves against Amblyseius mackenziei Muma (Bakker and Sabelis 1986). They are likely equally able to defend themselves against A. cucumeris. Pseudopupae drop from the plant and rest in the soil until emerging as adults, thus being absent from the foliage. Only first-instar nymphs are left as highly-acceptable prey. This high stage-limited host specificity is an undesirable characteristic and reduces the ability of A. cucumeris to control F. occidentalis (DeKlerk and Ramakers 1986).

Finally, <u>A</u>. <u>cucumeris</u> shows a strong numerical response to <u>F</u>. <u>occidentalis</u> populations, at least in the absence of pesticides. Its short life cycle in relation to WFT allows it to overtake a burgeoning prey population. A functional response may also be present in <u>A</u>. <u>cucumeris</u>. Density-dependent feeding has been observed in <u>Amblyseius degenerans</u> Berlese and other phytoseiids (Eveleigh and Chant 1981), manifesting itself as diminished feeding activity at low prey density, and reduced consumption per prey individual as prey density increases.

E. SUMMARY AND CONCLUSIONS

This study has demonstrated the ability of <u>A</u>. <u>cucumeris</u> to control <u>F</u>. <u>occidentalis</u> in cucumber greenhouses. In 2 cases, control of WFT was successful. In one of these, early establishment of <u>A</u>. <u>cucumeris</u> led to season-long suppression of WFT . In the other, an early infestation was eventually controlled. In the third case biological control failed after applications of pesticides began. Success may depend upon avoiding pesticide use. Of the few pesticides which are not acutely toxic, sublethal effects of reduced longevity, fecundity, developmental rates, or altered behaviour are likely. These sublethal effects may have been responsible for the breakdown of biological control in the "successful" cases following fungicide applications in September. The other factors which may have been responsible for the breakdown of biological

control were short daylength, low RH, and low night temperatures.⁷

The effect of temperature is being investigated by D. R. Gillespie, Agriculture Canada, Agassiz, B.C.

CHAPTER 3: DAYLENGTH STUDIES

A. INTRODUCTION

Daylength is a common cue used by insects in the induction of diapause (Borror et al 1981). Although some respond to long daylength, most enter diapause as a result of short daylength. Diapause is triggered by a specific length of photophase, the critical daylength, which varies among insects (Chapman 1969). Temperature often interacts with daylength in diapause induction, but progressive changes in daylength are rarely involved with diapause induction in insects.

Other phytoseiids, notably <u>Amblyseius fallacis</u> Garman (Swift 1987) and <u>Typhlodromus occidentalis</u> Nesbitt (Croft 1971) undergo daylength-induced diapause. The critical daylengths were just under 12 h and between 13 and 15 h, respectively. The most sensitive stages to daylength are the eggs and the larvae; reproductive adults placed in short daylength do not enter diapause (Hoy 1975; Swift 1987).

The following experiments were designed to determine whether daylength has an effect on the development, longevity, and fecundity of <u>A</u>. <u>cucumeris</u>. Because only 3 light regimes were used, the critical daylength was not determined.

B. MATERIALS AND METHODS

Three light regimes were used: 16:8 (L:D), 12:12, and 8:16. These regimes encompass the longest and shortest days encountered under growing conditions. Day and night temperatures were 22° and 18°C, respectively, representative for early spring and late fall, when <u>A</u>. <u>cucumeris</u> performs poorly.

A. cucumeris were reared in bran (Ramakers and van Lieburg 1982). Bran mites, <u>Acarus farris</u> Oudemans, were raised on a mixture of bran, brewer's yeast, and other nutrients in an airtight tub through which humidified air was forced. When the density of <u>A. farris</u> was sufficiently high, the culture was removed to a separate tub and an inoculum of <u>A. cucumeris</u> in bran from an old culture was layered on top. The tub was sealed and humidified air was forced through the mixture. <u>A. cucumeris</u> fed and multiplied on all stages of <u>A. farris</u>. When the density of <u>A. cucumeris</u> peaked, some of the culture was set aside as a new inoculum; the rest was used for experimentation.

i. Egg Hatch

One hundred eighty <u>A.</u> cucumeris eggs under 24 h old were collected by setting a 2 cm x 5 cm rectangle of black felt on top of the culture medium. The wool fibres are an attractive oviposition site for <u>A.</u> cucumeris. After one day, the felt was removed and the eggs gently brushed off into a glass petri dish.

Ten eggs were placed in each of 18 1.0 x 3.0 cm petri dish lids, painted black on the outside, and glued to the inside bottom of a 1.5 x 10.0 cm petri dish. Saturated KCl solution was poured around the small dish to maintain a static humidity of 85% within the closed, larger dish (Richardson and Malthus 1955). Eggs were placed in the small dish, and the apparatus was covered to form an airtight chamber. Six dishes were subjected

to each light regime. Temperatures within the dishes were less than 1°C different than those outside the dishes. Numbers of hatched eggs were counted daily until 100% hatch or for 1 week, whichever came first. Data were analyzed with a 2-way ANOVA (factors were daylength and day after start), followed by a 1way ANOVA for each day's hatch (Zar 1984).

ii. Developmental Stages

Five pinto bean leaf discs (2 cm diam.) were arranged on cotton batt (0.5 cm thick) soaked with distilled water in square petri dishes (1.0 x 15 x 15 cm). The water kept the leaf disc alive and provided a moat to prevent arthropods from escaping. A 0.5 x 0.5 cm square of 0.2 cm thick clear acetate sheet was laid upon each disc to provide a thigmotactic refuge for \underline{A} . cucumeris, effectively eliminating escapes. One A. cucumeris eqq (≤ 24 h old) was set on each leaf disc; 10 discs (2 petri dishes) were used in each treatment. After eggs hatched, larvae and protonymphs were fed coddled (heated in 50°C water until all movement ceased, ca. 15 minutes) second instar thrips nymphs. Deutonymphs were fed live, 1-day-old, first instar thrips nymphs. Days to eclosion, to first moult (protonymph), to second moult (deutonymph), and to third moult (adult) were noted. Moults were determined by the presence of exuviae, which were easy to see on the leaf discs. Developmental time for each instar and days from eclosion to adult were analyzed using 1-way ANOVA.

iii. Adult oviposition and longevity

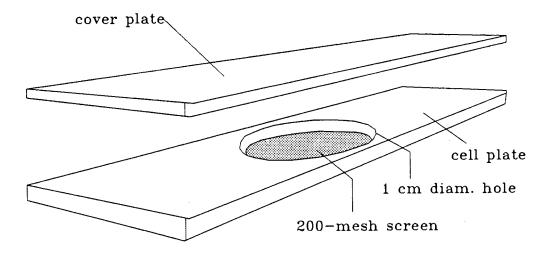
A. <u>cucumeris</u> were reared on leaf discs maintained as in the developmental studies. Ten eggs were placed on each leaf disc; one set of 5 discs (one petri plate) was set under each daylength regime. After hatch, <u>A. cucumeris</u> were fed unlimited coddled second instar WFT nymphs. Old thrips nymphs were removed each day and new ones added. The first day mating adults were observed, they were discarded in the interests of collecting even-aged adults. Discards amounted to 5 - 10% of the total. Females collected beyond this point were enclosed in an experimental cell with adult males. The following day, mating was assumed to have taken place, and females were used in experiments.

Experiments utilized modified Munger cells (Munger 1942) (Fig. 3). Holes (1 cm diam.) were drilled in 0.1 x 2 x 5 cm rectangles of plexiglass; fine-mesh screen (200 mesh) was glued across one side of each hole. An intact rectangle of plexiglass was positioned over the unscreened side of the hole and held in place with 2 clothespins, creating a 0.1 cm deep arthropod-proof chamber which would allow maximum air exchange.

One mated <u>A</u>. <u>cucumeris</u> female was placed in each of 30 cells; 10 cells were placed in each of 3 8 x 14 x 18 cm airtight chambers. Cells rested on a plastic grid 1.5 cm above the chamber bottom. Saturated KCl solution was poured to a depth of 0.5 cm in the chamber to maintain a RH of 85%. One chamber was subject to each light regime, resulting in 10 isolated mated <u>A</u>. <u>cucumeris</u> females under each light regime. Temperatures within

Figure 3. Modified Munger cell. Plexiglass plates are held together with clothespins.

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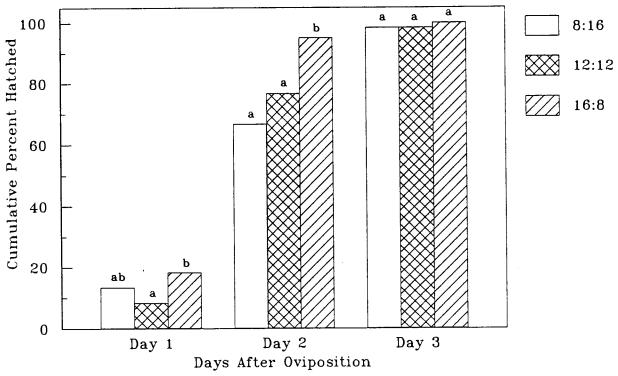
the chambers were less than 1°C different than those outside the chambers. Females were fed 4-6 live 1st-instar WFT nymphs or 3-5 coddled second instar WFT nymphs daily, depending upon availability. All replicates received the same prey on any given day. Day-old and eaten nymphs were removed at feeding. Numbers of eggs laid per day and longevity were recorded until all <u>A</u>. <u>cucumeris</u> had perished. Data for total eggs laid, for eggs laid per day, and for longevity were analyzed by 1-way ANOVA.

C. RESULTS AND DISCUSSION.

i. Egg Hatch

Few eggs hatched on the first day after oviposition (Fig. 4). On the second day, there was a significantly more rapid hatch under the 16:8 than under the 8:16 and 12:12 (L:D) regimes. By the end of the third day, all groups had hatched successfully.

The early hatch under long-daylength (16:8) conditions may have been an artifact of higher daytime temperatures, leading to more accumulated day-degrees at longer daylengths. However, since the non-feeding larvae are little more than eggs with legs, there may be some advantage to early motility under longdaylength (16:8) conditions. Possibly egg predation pressure is highest under longer-daylength conditions, thus selecting for early motility. Or possibly the quantity or quality of <u>A</u>. <u>cucumeris</u> prey is different during longer-daylength regimes than Figure 4. <u>A</u>. <u>cucumeris</u> egg hatch under different daylengths. Bars topped by the same letter are not significantly different (Newman-Keuls Test, α <.05).



during short-daylength (8:16) regimes, giving an advantage to earlier development of feeding stages (protonymphs).

Regardless of the rate of hatch, final hatch under all daylengths was the same. Thus, there appears to be some sort of light detection by eggs which determines not whether, but how fast, eggs will hatch. Although postponed hatching (slower hatch rate) might increase an individual's fitness, not hatching cannot possibly do so; it would make little ecological sense to stop egg hatch under certain daylengths.

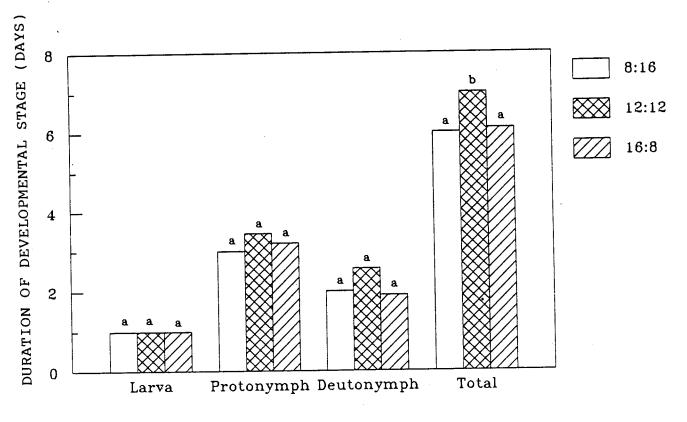
ii. Developmental Rates

No significant differences were found for the developmental rates of individual stages between daylengths (Fig. 5). However, the total time elapsed from hatching to adulthood was ca. 15% longer under mid-daylengths than under either short or long daylengths. This result appears at first to contradict the egg hatch data, but once an egg has become motile (possibly to avoid predation pressure), other ecological concerns may take precedence. However, for all practical purposes, the 15% difference in developmental rates under different daylengths may be biologically insignificant, and might even be an artifact of the experimental procedure.

iii. Adult Oviposition and Longevity

No significant differences were found between daylengths for total eggs laid, eggs laid per day, or longevity of adults (Table 2). Thus daylength had no demonstrable effect on the

Figure 5. Relationship between photoperiodic regime and duration of developmental stage in <u>A</u>. <u>cucumeris</u>. Bars topped by the same letter are not significantly different (Newman-Keuls test, α <.05).



DEVELOPMENT STAGE

TABLE 2. Oviposition and longevity of adult female <u>A</u>. <u>cucumeris</u> under different daylengths.

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PHOTOPERIOD (light:dark)	MEAN EGGS/DAY (mean ± S.E.) ^a	TOTAL EGGS (mean ± S.E.) ^a	LONGEVITY (mean ± S.E.)	
16:8	0.59 ±0.034	14.8 ±2.01	24.8 ±3.36	
12:12	0.57 ±0.027	16.8 ±1.64	29. 9 ±2.74	
8:16	0.52 ±0.034	15.0 ±2.01	29.2 ±3.36	

^aNo significant differences between any means within a column (ANOVA, α >.05).

reproductive performance of <u>A</u>. <u>cucumeris</u>. Reproductive diapause was not induced by any specific daylength, as evidenced by no decrease in fecundity, and no increase in longevity.

D. CONCLUSIONS

The entire life cycle of <u>A. cucumeris</u> has been tested under three daylengths, from egg hatch through oviposition to death. Although rate of egg hatch increased with daylength, and total development time was highest at an intermediate daylength, the actual differences between treatments were small and may have no biological meaning.

These experiments demonstrate that daylength alone likely was not responsible for the poor performance of <u>A</u>. <u>cucumeris</u> in early spring and fall in commercial greenhouse studies (Fig. 1). Daylength may still, however, be an integral part of the performance of <u>A</u>. <u>cucumeris</u>. Predators may respond to changing daylengths, rather than static conditions as tested here. They may also respond to a combination of daylength and some other growth factor, such as temperature. These hypotheses, though beyond the scope of this thesis, must be tested before a complete understanding of the capability of <u>A</u>. <u>cucumeris</u> as a biological control agent is possible.

CHAPTER 4: RELATIVE HUMIDITY

A. INTRODUCTION

Low relative humidity (RH) is has a detrimental effect on the ability of <u>P</u>. <u>persimilis</u> to control <u>T</u>. <u>urticae</u> in greenhouses (N. W. Hussey,⁸ pers. comm.). <u>Amblyseius idaeus</u> and <u>A</u>. <u>anonymous</u> are adversely affected by low RH in both egg hatch and performance of motile stages (Dinh et al 1988). Although <u>A</u>. <u>idaeus</u> can surviving RH as low as 30%, <u>A</u>. <u>anonymous</u> and most other phytoseiids are unable to withstand RH below ca. 60%. My objective was to determine if a similar humidity response exists in <u>A</u>. <u>cucumeris</u>.

B. MATERIALS AND METHODS

Five RH's were used: 95%, 85%, 70%, 55%, and 40%; 100% RH (pure distilled water) was not used due to condensation problems. RH was controlled statically by the use of salt solutions. Air contained above a salt solution will attain an equilibrium RH depending upon the vapour pressure of the water in solution (Richardson and Malthus 1955). This vapour pressure and the resulting RH depend on which salt is used and the concentration of the solution.

One method of humidity control utilizes saturated salt or glucose solutions, which are in contact with excess solid (Winston and Bates 1960; Richardson and Malthus 1955). These

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solutions are stable, and can be stored and re-used readily. They are also stable with regard to water loss or gain from the air above them. If water is lost, salt or glucose crystalizes out of solution, so that the solution remains saturated; if water is gained the reverse happens. Because of this feature, standardized RH's can be achieved. Saturated salt solutions were used for a preliminary set of experiments. However, because of suspected toxicity problems and differences between theoretical and actual (measured) RH, it was abandoned for the second method.

A second method uses unsaturated solutions of CaCl₂ (Stokes and Robinson 1949). These solutions are readily prepared and the equilibrium RH's are more temperature stable than with the saturated-salt method, but are less stable with regard to water loss or gain. Any water gained or lost from solution results in a change in concentration, thus changing the solution's vapour pressure and the RH in the air above. To avoid this problem, the solution was used in large excess (Richardson and Malthus 1955). Since the arthropods used were minute compared to the bulk of solution used, and because the chambers were open for less than 1 min per day, changes in concentration of the solutions were considered to be negligible. Nonetheless, solutions were changed weekly in experiments lasting >1 week.

Concentrations of CaCl₂ (w/w in distilled water) used are as follows (RH in parentheses): 9.33% (95%); 19.03% (85%); 27.4% (70%); 33.71% (55%); 39.62% (40%).

The rate of equilibration of RH within closed chambers is rapid. Marcandier and Khachatourians (1987) found the RH in large empty vessels re-equilibrated within 1-2 h after being opened for 3 min. Measurements of the RH in the chambers used in this study indicated that approximate equilibration occurred after just 30 min. Within another hour the RH was stable (Fig. 6). The speed of equilibration is increased as the height of the air column above the salt solution decreases; for this reason small (8 x 14 x 18 cm) containers were used.

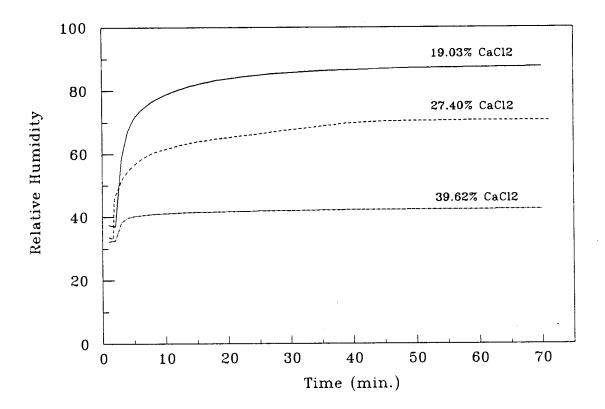
Four experiments were conducted to determine the effects of RH on <u>A</u>. <u>cucumeris</u> performance. In each experiment, 200 ml of each of the 5 solutions were placed in the bottom of an airtight container, for a total of 5 containers. Modified Munger cells (Fig. 3) containing arthropods were set on a plastic grid which held them 1 cm above the salt solution.

Temperatures were maintained in a growth chamber at 24 to 25°C for studies on oviposition and longevity, or on a lab bench at 19 to 20°C for studies on egg hatch and developmental rates. Ambient RH ranged from 33 to 38%. Daylength was set at 16:8 L:D in the growth chamber, and averaged the same in the laboratory.

i. Egg Hatch

New eggs (≤ 24 h old) were collected from an active <u>A</u>. <u>cucumeris</u> culture as described in Chapter 3. Five eggs were placed in each of 25 modified Munger cells; 5 cells were placed in each of the 5 RH chambers. Hatched eggs were counted daily

Figure 6. RH stabilization within an enclosed chamber used for determining the effects of RH on <u>A</u>. <u>cucumeris</u>. Chambers were left open (ambient RH= 33-37%) until min 3, then closed.



until all eggs had hatched or for 1 week, whichever came first. Data were analyzed with a 2-way ANOVA for the entire data set, followed by a 1-way ANOVA for each day's hatch.

ii. Developmental Stages

Fifty neonate larvae were collected from the egg hatch study above. Each was placed in a modified Munger cell; 10 cells were enclosed in each of the 5 RH chambers. Larvae and protonymphs were fed daily with coddled second instar WFT nymphs. Deutonymphs were fed either coddled second instar or live first instar WFT nymphs, according to availability. On any given day, mites in all replicates were fed the same type and quantity of prey. All day-old prey, consumed or otherwise, were removed when new prey were introduced. Days to first moult (protonymph), to second moult (deutonymph), and to third moult (adult) were noted. When possible, the presence of exuviae was used to determine when moulting between protonymph and deutonymph had occurred. Otherwise, morphological changes were The duration of each stadium and total developmental time used. were analyzed separately by 1-way ANOVA. Differences in rates of death between treatments were analyzed by multiple Chi-square analysis. Because of high juvenile mortality, replicates were included in the analysis for each stage only if the mites survived that stage and moulted to the next.

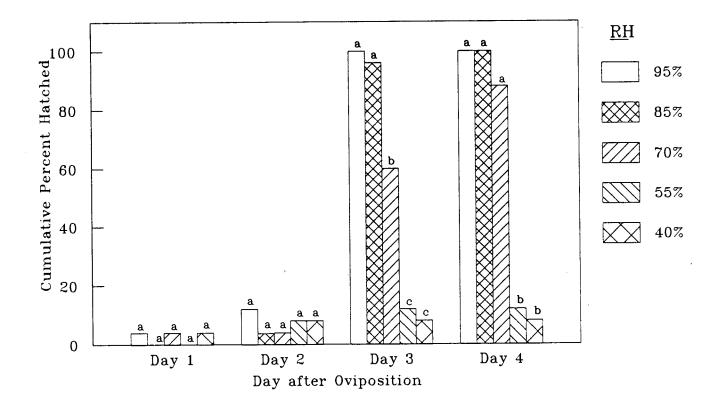
iii. Adult oviposition and longevity

Even-aged, mated, female <u>A. cucumeris</u> were reared as described in Chapter 3. One mated female was placed in each of 50 modified Munger cells. Ten modified Munger cells were enclosed in each of the 5 RH chambers. Each female was fed daily with 4-6 live 1st-instar WFT nymphs, or 4-5 coddled second-instar WFT nymphs. On any given day, all predators received the same prey. Day-old and consumed WFT nymphs were removed at feeding. Numbers of eggs laid were recorded daily, except for the first 2 days, which were treated as an adjustment period. Each trial continued until all <u>A. cucumeris</u> had died. Total eggs laid, eggs per day, and longevity were recorded. Data were analyzed with 1-way ANOVA followed by linear regression analysis of pooled 85 + 95% verses pooled 55 + 40% data (Zar 1984).

C. RESULTS AND DISCUSSION

i. Egg Hatch

A. <u>cucumeris</u> eggs hatched at a significantly more rapid rate at high RH (95% and 85%) than at lower RH (55% and 40%) (Fig. 7). This differential hatch rate is probably related to high egg mortality and retarded development in lower RH's. The rate of egg hatch may affect overall performance throughout a predator's life. Even if the predator fully recovers from exposure to low RH, its development has been slowed, thus reducing its potential rate of increase. Figure 7. <u>A</u>. <u>cucumeris</u> egg hatch under different RH regimes. Bars within each day topped by the same letter are not significantly different (Newman-Keuls test, $\alpha < .05$).



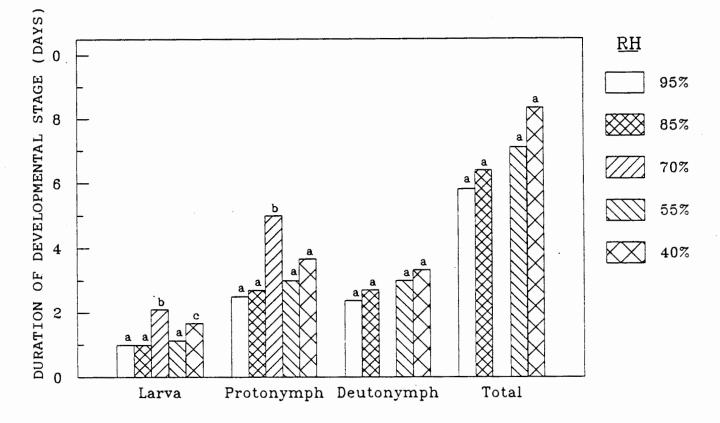
The threshold RH for successful hatch seems to be approximately 70%, at which intermediate hatch rates were observed (Fig. 7). Below this level, few eggs hatched; unhatched eggs became visibly desiccated after 3 days' exposure to 45 or 55% RH. Those that did hatch did so within 2 days of oviposition. Thus eggs can withstand short periods of exposure to low RH, but die under constant exposure. Since RH in the greenhouse fluctuates on a diurnal cycle, RH might not play as crucial a role as suggested by this experiment. Periodic depressions in RH may slow the rate of egg hatch, however.

ii. Developmental Stages

The time required for larvae to develop was highly affected by RH (Fig. 8). However, developmental time was not linearly related to RH. The time required for development at 55% was not significantly different than that at 85% or 95%; the time at 70% was significantly longer than that at 40%.

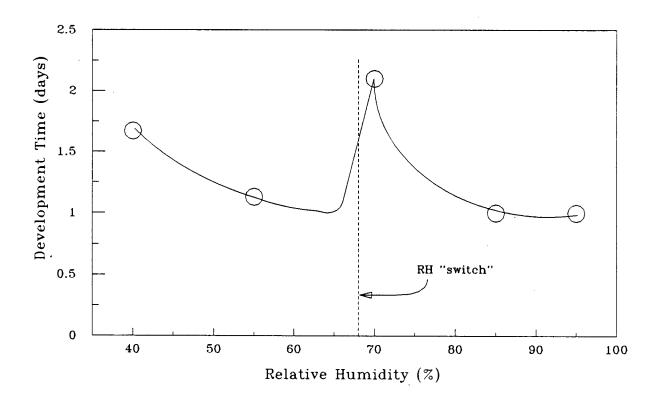
I hypothesize that these results may be the product of a RH "switch" (Fig. 9). Larvae may be sensitive to low RH because of a thin cuticle or because they are non-feeding, thus unable to replenish lost water. The lower the RH, the longer the developmental time, until at some low RH, development would cease and the larva would die. In this case, early moulting to the protonymph stage would be an advantage under low RH's. If the switch to early moulting was made at an RH somewhere below 70%, then developmental time would increase with decreasing RH until 70%. Developmental time would initially drop as RH

Figure 8. Relative humidity and developmental stage. Bars within each stage topped by the same letter are not significantly different (Newman-Keuls test, α <.05).



DEVELOPMENTAL STAGE

Figure 9. Hypothesized RH "switch" for larval development time of <u>A</u>. <u>cucumeris</u>.

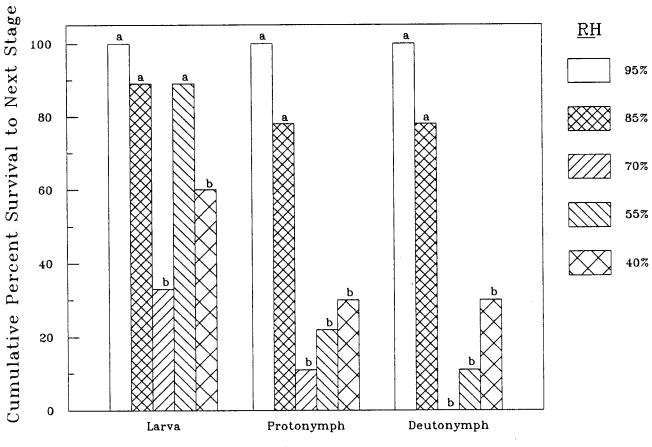


declined below 70% (crossing the switch), then continue to increase with decreasing RH.

The development of protonymphs was also highly affected by RH (Fig. 8). However, only at 70% RH was development significantly longer than at the other RH's; no significant differences in developmental time were detected between any of the other RH's. At first this trend seems to support the response of larvae to RH. However, only 1 replicate (1 mite) in the 70% regime survived the protonymph stage (Fig. 10); it was severely dehydrated after moulting to protonymph and died after moulting deutonymph. This mean is, therefore, of doubtful value. If it is discarded, it can be concluded that RH has no significant effect on the development of protonymphs. Similarly, there was no effect of RH on the development of deutonymphs.

Although mites in many replicates in the lower RH's survived the larval stage, they died either when moulting to protonymphs or shortly thereafter (Fig. 10). Those that survived the protonymph stage all appeared slightly dehydrated after moulting from larvae to protonymphs, but appeared normal by the end of the stadium. Thus it appears that if a predator can survive the larval stage and first moult at low RH , it will recover in later stages. This conclusion is supported by the overall developmental times (Fig. 8). Although there was a trend toward longer development at lower RH, this trend was not significant, suggesting that not only will predators recover if they survive the larval stage and first moult, but will catch up to their cohorts in higher RH.

Figure 10. Mortality of <u>A</u>. <u>cucumeris</u> in different RH's. Each stage was analyzed seperately with multiple Chi-square analysis, α <.05.





Mortality of juvenile <u>A</u>. <u>cucumeris</u> again exhibited an RH threshold. Two significantly different groupings emerged by the end of the deutonymph stage (Fig. 10): at 95 and 85% RH there were few deaths; there was >80% combined mortality at 70, 55, and 40% RH.

iii. Oviposition and Longevity

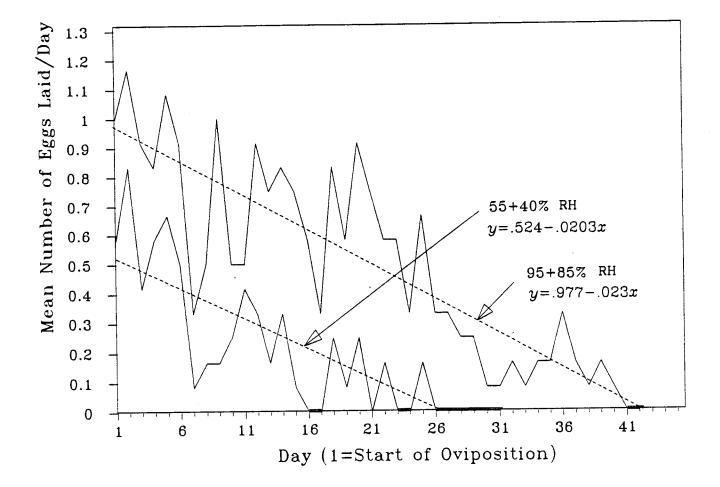
There was no significant difference in the daily oviposition between RH's (Table 3). However, significantly higher total oviposition was found in the 2 highest RH's compared to the 2 lowest. There was also a significant decline in longevity as RH decreased.

These results can be better understood with regression analysis. If the data for the 2 highest RH's are pooled by taking a grand mean across each day for both RH's, and the lowest 2 RH's are similarly pooled, 2 regression lines can be drawn (Fig. 11). Under both RH regimes, the daily oviposition was strongly related to age $(r^2=.7004, p<.01; r^2=.6368, p<.01$ for high and low RH, respectively). The slopes between the regression lines were not significantly different, but there was a highly significant difference between y-intercepts. Overall fecundity (the area under each line) was suppressed at low RH. Daily egg production (total eggs divided by days lived) was similar (Table 3), but at any given time, the predators at low RH were only half as fecund as those at high RH. Thus <u>A</u>. <u>cucumeris</u> appears to utilize some of its water resources for purposes other than egg production at low RH. This in turn must

RELATIVE HUMIDITY	MEAN EGGS/DAY (mean ± S.E.)	TOTAL EGGS (mean ± S.E.)		LONGEVITY (mean ± S.E.)	
95%	0.54 ±0.12a	16.5	±2.14a	33.8	±4.54a
85%	0.65 ±0.13a	15.2	±2. 35a	28.6	±4.96ab
70%	0.67 ±0.13a	9.8	±2.35a b	17.0	±4.96 k
55%	0.67 ±0.12a	8.7	±2.14a b	18.5	±4.53 k
40%	0.67 ±0.13a	5.2	±2.35 b	13.8	±4.96 k

TABLE 3. Oviposition (total and daily mean) and longevity of adult female <u>A. cucumeris</u> under different RH regimes.

Means within a column followed by the same letter are not significantly different (Newman-Keuls Range Test for Mean Eggs and Total Eggs; Duncan's Multiple Range Test for Longevity, α <.05) Figure 11. Relationship between daily egg production and adult age for <u>A</u>. <u>cucumeris</u> under high and low RH regimes.



affect the long-term ability of <u>A</u>. <u>cucumeris</u> to suppress populations of WFT.

D. CONCLUSIONS

The experiments described in this chapter demonstrate the severe adverse effect of low RH on the development and performance of <u>A</u>. <u>cucumeris</u>. At low RH, eggs fail to hatch, juveniles die, and adults live less time and lay fewer eggs. Because of the increased death rate and reduced oviposition, it can be claimed that r, or the intrinsic rate of increase (Pianka 1983) of <u>A</u>. <u>cucumeris</u>, is reduced when exposed to RH under 70%, affecting its usefulness as a biological control agent.

As discussed in Chapter 2, <u>A</u>. <u>cucumeris</u> has several shortcomings as a biological control agent. One of its strong points is its short life cycle and high fecundity, i.e. it has a higher r than WFT. In low RH, though, r declines rapidly in <u>A</u>. <u>cucumeris</u>, severely limiting its effectiveness as a biological control agent. At the same time, WFT may be less affected by low RH than <u>A</u>. <u>cucumeris</u>, because of the ready availability of succulent leaf tissue. This may explain, at least in part, the poor control of WFT observed in early Spring and Fall in the commercial greenhouse trials.

These experiments were performed under absolute RH conditions. In the greenhouse, however, RH not only fluctuates, but the dense foliage creates pockets of dead air which may have higher or lower RH than ambient. Also, the surface of each leaf has a boundary layer of dead air. This layer is especially thick and influential under low wind conditions (Salisbury and Ross 1978) such as found in greenhouses. As boundary layer thickens, RH next to the leaf surface is increasingly less affected by ambient RH. Therefore even under low ambient RH, the actual RH surrounding leaf-surface arthropods may not be inhibiting.

Despite the difficulties in extrapolating these laboratory experiments to the greenhouse, they provide a rigorous means of determining the effects of RH. Since greenhouse growers routinely monitor ambient RH, further studies may easily be conducted to determine whether RH is important under greenhouse conditions.

CHAPTER 5: CONCLUSIONS

The studies presented in this thesis describe the rapid development of a biological control agent, <u>A</u>. <u>cucumeris</u>, against a new greenhouse pest, <u>F</u>. <u>occidentalis</u>. The predator successfully controlled its prey on 2 of 3 farms, in all but early spring and fall. It is concluded that the main reason for failure on 1 farm was the extensive use of pesticides.

Three hypotheses were put forth to explain early and lateseason failures. The effect of low temperatures was not studied here because others undertook this work. The effects of daylength and RH were studied.

Daylength had no effect on the life history of <u>A</u>. <u>cucumeris</u>. Although a slightly faster rate of egg hatch and slower developmental rate under a 12:12 h L:D regime was detected than at 16:8 or 8:16 h regimes, the differences were insufficient to affect the life history of <u>A</u>. <u>cucumeris</u>. One might tentatively conclude that the performance of <u>A</u>. <u>cucumeris</u> as a predator of WFT will be unaffected by photoperiod. However, other researchers have since determined that diapause in <u>A</u>. <u>cucumeris</u> is induced by the combined action of daylength and temperature (L. Gilkeson,⁹ pers. comm.). Two possibilities for circumventing the problem exist: breeding for diapause-free <u>A</u>. <u>cucumeris</u> and adding light to greenhouses to increase the photophase. The latter method is used with <u>Aphidoletes</u>

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<u>aphidimyza</u> Felt, a commercially available aphid biological control agent (Gilkeson and Hill 1986). Critical light or dark periods, critical light intensity, and temperature interactions would have to be established before this method becomes practical.

Finally, it was discovered that RH plays a significant role in the development and performance of <u>A. cucumeris</u>. A critical threshold for RH at approximately 70% was apparent, below which eggs failed to hatch, juvenile mortality increased, and adult longevity and fecundity were reduced. The effect of RH was tested under constant absolute RH conditions which did not assess the importance of fluctuating RH's or the boundary humidity layer on leaf surfaces. Therefore, new experiments must be run on the leaf surface under greenhouse conditions, or the RH at the leaf surface must be measured to determine its effect at different ambient RH's. Moreover, further work on fluctuating RH's must be done before the effects of RH on <u>A. cucumeris</u> in the greenhouse are fully understood.

It was apparent that considerable natural variation exists in the response of <u>A</u>. <u>cucumeris</u> to RH. Thus it may be possible to selectively breed <u>A</u>. <u>cucumeris</u> for low RH tolerance. The selection for tolerance to low RH, in combination with other factors such as pesticide resistance and lack of diapause, could create a powerful and easily used biological control agent.

In conclusion, biological control of WFT by <u>A</u>. <u>cucumeris</u> could be improved by a better understanding of the life cycle, microhabitat preference, and feeding habits of the predator. Knowledge of optimal timing and rate of release in relation to \underline{F} . occidentalis infestations could be used to improve the efficiency of a commercially implemented system. Finally, the discovery or selection of strains of \underline{A} . cucumeris tolerant to pesticides and low RH, and without diapause would result in a more robust and easily integrated biological control system.

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