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Title: MECHANISMS OF HOP RESISTANCE TO THE TWOSPOTTED SPIDER MITE (TETRANYCHUS URTICAE KOCH)

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Ralph E. Berry

Hop varieties were compared under field and greenhouse conditions to determine their relative susceptibility to the twospotted spider mite, Tetranychus urticae Koch. A fertility schedule was prepared for mites on resistant and susceptible varieties, and tests were conducted to compare sex ratio, oviposition rate, survival rate, and developmental rate of mites on resistant and susceptible varieties. Hop leaves were examined for traits that could affect mite life histories. Moisture content of leaves, leaf area, and density of ventral glands and hairs were related to oviposition, sex ratio, developmental times, survival, and female migration, using linear regression. Thin layer chromatography (TLC), column chromatography, and gas-liquid chromatography (GLC) were used to analyze farnesol content of hop foliage and oil. Farnesol, nerolidol, geraniol, nerol, and an ether extract of twospotted spider

mites were topically applied to female mites to determine effects on oviposition rates.

In the greenhouse, mite densities were significantly greater ($P \leq 0.05$) on Comet and Fuggle than on L-8, L-1, and Cascade, using artificially infested plants. In the field, natural infestations were significantly lower ($P \leq 0.005$) on Fuggle and Cascade than on Talisman or Comet, and L-16 had the highest density.

Mites on Cascade had a higher intrinsic rate of natural increase than on Fuggle, Comet, Talisman, or L-16, and mites on Cascade had the shortest generation time. The net reproductive rate was higher on Fuggle than on Cascade, Comet, Talisman, or L-16.

No consistent differences were found in oviposition rates, sex ratio, or survival of mites reared on resistant and susceptible varieties. Highly significant differences ($P \leq 0.005$) were found in developmental rates, and the developmental rate of mites on L-16 was consistently slowed. Immature mites on L-16 and Talisman developed slowly, and differed in coloration from mites on Cascade, Fuggle, and Comet.

Leaves from Cascade, Comet, L-16, and Talisman differed significantly ($P \leq 0.005$) in moisture content and density of ventral hairs and glands. Statistically significant regressions were obtained relating oviposition to leaf area, sex ratio to ventral hairs,

and development time to leaf moisture content and ventral hairs.

Migrating females preferred Comet leaves to leaves from Talisman, Cascade, Fuggle, and L-16; preference was not significantly related to leaf area, ventral hair density, ventral gland density, or leaf moisture content.

TLC and GLC methods were developed for detecting farnesol in hop foliage. Farnesol was detected in hop oil obtained from Fuggle cones. No farnesol was detected in petroleum ether extracts of hop foliage, using detection levels ranging from 6-200 μg farnesol/g of dry foliage.

Topical applications of farnesol, nerolidol, geraniol, nerol, and mite extract produced no consistent effect on mite oviposition. Farnesol does not appear to be related to the resistance of hops to the twospotted spider mite, and it does not appear to act as a gonadotropin.

Mechanisms of Hop Resistance to the Twospotted Spider Mite (*Tetranychus urticae* Koch)

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MECHANISMS OF HOP RESISTANCE TO THE TWOSPOTTED SPIDER MITE (TETRANYCHUS URTICAE KOCH)

I. INTRODUCTION

The twospotted spider mite, Tetranychus urticae Koch, is a major pest of hops in the Pacific Northwest. Outbreaks are most severe in areas such as central Washington, southwestern Idaho, and southern Oregon, where hot, dry conditions encourage mite reproduction. Foliar damage weakens the hop plants, and feeding injury on the cones reduces the market value of the crop.

Growers in the Pacific Northwest use systemic and contact acaricides to reduce mite infestations, but economic damage often occurs in spite of control attempts. Additional problems are anticipated because mites can develop resistance to pesticides, and because pesticides are subject to changes in registration status. For these reasons, researchers are searching for control measures which can be used in addition to, or in lieu of, chemical controls.

In many crops, the development of resistant varieties has assisted pest control. If a hop variety which is resistant to mites could be developed, it might increase the growers' ability to keep mite damage under economically damaging levels.

The purpose of this study is to confirm previous reports (Mayberry 1968, Regev and Cone 1975b) that hop varieties differ in

susceptibility to the twospotted spider mite, and to investigate the mechanisms by which hop varieties may resist spider mite infestations.

II. LITERATURE REVIEW

The Twospotted Spider Mite

The twospotted spider mite, Tetranychus urticae Koch, is a pest of many crops. Its increasing importance as an agricultural pest is attributed to the mite's ability to develop resistance to acaricides (Pfadt 1962), to the adverse effects of non-selective pesticides on predator populations, and to agricultural practices which have improved the nutritional quality of the host plants (van de Vrie et al. 1972).

Tetranychus bimaculatus (Harvey) and T. telarius (Linn.) are synonyms of T. urticae (Mayberry 1968). T. telarius is now used to designate a separate species. T. cinnabarinus (Boisduval) was thought to be the same as T. urticae, but is now recognized as a distinct species (van de Vrie et al. 1972).

The twospotted spider mite is a member of the family Tetranychidae, which contains many important crop pests. Tetranychids have a life cycle consisting of five stages. The egg hatches into a six-legged larva, which molts into an eight-legged protonymph. All subsequent stages have eight legs. A second molt produces the deutonymph, which is larger than the protonymph, but otherwise similar in appearance. The deutonymph molts into the adult stage. The larval stage and both nymphal stages begin with

an active period in which the mite feeds, followed by a quiescent stage which ends in the molt (van de Vrie et al. 1972).

Twospotted spider mites reproduce throughout the year in some areas, but in northern regions diapausing females are the only overwintering stage. Declining daylength is the most important stimulus for initiating diapause, but declining temperatures and poor food supplies are also influential. Increasing temperatures and food availability terminate diapause in the spring (van de Vrie et al. 1972). In Oregon hop yards, mites overwinter on the basal leaves of the hop vines, in the soil, or on nearby vegetation, and resume activity in spring or early summer (Mayberry 1968).

The life cycle of the twospotted spider mite is very short. The developmental rates reported in the literature vary; Mayberry (1968) reported an average developmental time from egg to adult of ca. 10 days, while Laing's cultures required more than 16 days to complete development (Laing 1969), and Mitchell (1973) reported a time of ca. 17 days. Developmental rates are influenced by temperature, humidity, and food quality (van de Vrie et al. 1972). Males develop more rapidly than females (Laing 1969).

Quiescent female deutonymphs attract males, which attend the female until she emerges as an active adult (Cone et al. 1971). Mating usually occurs immediately after the female emerges (Laing 1969). A short preoviposition period follows emergence.

Averages of 1.8 days (Mayberry 1968) and 2.1 days (Laing 1969) have been reported.

Reports of twospotted spider mite fecundity and oviposition periods vary. Laing (1969) reported an average oviposition period of 15.7 days, and an average total egg production per female of 37.9 eggs. Mayberry (1968) reported an average of 101.3 eggs per female, with a mean oviposition period of 17.4 days.

Unmated females lay unfertilized eggs, which hatch into males. Mated females produce male and female progeny, and the females usually outnumber the males (van de Vrie et al. 1972).

Hop Production

Hops, Humulus lupulus L., are dioecious perennial vines of the family Cannibinaceae. The native distribution of hops includes portions of North America, Europe, eastern Asia, and Japan (Burgess 1964). Most of the world's commercial crop is produced in England, Europe, and the United States. Most of the American crop is grown in the Pacific Northwestern states. Washington leads production with 17,833 kg/yr, followed by Oregon with 4087 kg/yr and Idaho with 2765 kg/yr (means for 1974-76, USDA 1977).

Commercial hop fields are established from rhizome cuttings, and remain productive for about 15 years. The vines, which die

back to the rootstock each fall, are trained on twine attached to an overhead wire trellis (Fig. 1). In the United States, trellises are usually about 5.5 m high, and are designed to facilitate machine harvesting (USDA 1961). The cones, which develop from female flowers, are harvested about three to four weeks after flowering. Flowering occurs from early to mid-summer, depending on the variety grown and climatic conditions (Burgess 1964). After harvest, the cones are dried and stored until sale.

Many hop varieties are used commercially. Some of the qualities selected in hop breeding are disease resistance, soil and moisture requirements, development time, brewing characteristics, and suitability for machine harvesting. Hop varieties differ in several morphological features, including the density of hairs and glands on the ventral surface of the leaves. They also differ in the amount and composition of the essential oils and resins produced by the cones. The genetic makeup of hops is complex, and a great deal of genetic diversity occurs within varieties (Burgess 1964).

Hop cones carry a large number of lupulin glands, which produce resins and essential oils. The resins, which are divided into hard and soft types, impart bitterness to beer. Alpha-acids and beta-acids are components of the soft resins; the alpha-acids are considered most important to the brewing quality of the cones. The essential oils, which give the beer aroma, include the monoterpene

Figure 1. Trellis system at the USDA hop breeding facility near Corvallis, OR.

Fig. 1

myrcene and the sesquiterpenes caryophyllene, farnesene, and humulene. The amounts of resin and essential oil obtained from the cones, and the relative proportions of their constituents, vary with variety, cultural conditions, and treatment of the cones during drying and storage (Burgess 1964).

Plant Resistance

Resistant plants are "inherently less damaged or less infested by a pest than others under comparable environments in the field" (National Academy of Science 1969). Painter (1951) described three types of insect resistance, which can also be applied to mite resistance: (1) nonpreference, which represents a plant's influence on the pest's host selection behavior; (2) antibiosis, a plant's adverse effect on the growth or survival of a pest; and (3) tolerance, a plant's ability to maintain vigor and yield in spite of infestation by a pest.

Resistance mechanisms may be physical or chemical (Southwood, 1973). Physical mechanisms, such as thickened cuticle or pubescent leaves, may prevent or interfere with a pest's attack on the plant. Chemical mechanisms of resistance are quite varied. Historically, resistance theorists have distinguished between plant compounds which act as nutrients, and secondary plant substances,

such as essential oils, tannins, and alkaloids, which are not known to play a nutritive role (Beck and Reese 1976).

Many studies on plant resistance to insects have focused on the role of secondary plant compounds. Particular interest has developed concerning plant substances which mimic, or are identical to, pheromones and hormones produced by insects. Some plants produce substances identical to insect pheromones. About 30 phytoecdysones (plant sterols which mimic ecdysone activity in insects) have been discovered, and two of them have structures identical to ecdysones produced by insects (Beck and Reese 1976). Juvabiones, which mimic insect juvenile hormone (JH), have been found in many plants (Beck and Reese 1976). Antiallotropins, substances produced by plants which inhibit JH by interfering with the corpora allata, have also been found (Bowers 1976).

Resistance of Crops to Tetranychus urticae

Plant resistance to the twospotted spider mite has been studied on many crops, including cotton, tobacco, tomatoes, strawberries, and cucurbits. Tolerance of mite damage has been reported in cotton (Schuster et al. 1972a), cucurbits (Rodriguez et al. 1975), and tomatoes (Rodriguez et al. 1972). Antibiosis occurs in cotton (Schuster et al. 1972b), cucurbits (Rodriguez et al. 1975), tobacco (Patterson et al. 1974) and tomatoes (Gentile et al. 1969).

Nonpreference has been noted in tobacco (Patterson et al. 1974) and cucurbits (Rodriguez et al. 1975).

The antibiotic effects noted in these studies include direct toxicity (Patterson et al. 1974), decreased fecundity (Gentile 1969, Schuster et al. 1972a, b, Patterson et al. 1974) and lengthened developmental rates (Schuster et al. 1972b).

Several plant characteristics are related to mite resistance, including hair density (Kishaba et al. 1972), the presence of glands (Schuster et al. 1972a), and glandular hair exudates (Patterson et al. 1974).

Biochemical characteristics have also been implicated in plant resistance to mites. Essential oils extracted from plants affect mites in several different ways. Toxic effects and repellent effects were noted with some fractions of essential oils extracted from tomatoes (Patterson et al. 1975). Some essential oil components from strawberry foliage were attractive to female mites at low concentrations, but became repellent at high concentrations, and some components acted as feeding stimulants or depressants (Rodriguez et al. 1976).

Previous research indicates that development of a hop variety resistant to spider mites may be feasible. Mayberry (1968) tested the fecundity of twospotted spider mites on ca. 300 genetic hop lines, and found differences in mite fecundity. Regev and Cone (1975b)

conducted greenhouse and field tests and demonstrated that hop varieties differed in susceptibility to twospotted spider mites. Berry (unpublished data)[1] conducted greenhouse and laboratory tests and confirmed varietal differences in susceptibility. He also obtained information which indicated that mite life histories are affected by different varieties.

Regev and Cone (1975b) suggested that the mechanism of hop resistance to T. urticae may be biochemical. They reported (1975a) that farnesol was found in extracts of quiescent female deutonymphs, and that commercially prepared farnesol attracted male twospotted spider mites. Regev and Cone (1975b) also analyzed extracts of hop foliage, and reported that farnesol was found in hop foliage, that its concentration varied with variety, and that varieties with high farnesol concentrations were susceptible to mites. They also stated (1976b) that topical applications of farnesol to female mites increased oviposition, and hypothesized that this could result from a juvenile hormone effect.

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[1] R.E. Berry, unpublished data on hop resistance to the twospotted spider mite. Oregon State University Entomology Department, Corvallis, OR.

III. COMPARATIVE RESISTANCE OF SELECTED HOP VARIETIES TO THE TWOSPOTTED SPIDER MITE

Introduction

The twospotted spider mite is cosmopolitan in its distribution and is a pest of many crops, including hops, Humulus lupulus (L.). Severe infestations develop in some hop-growing areas in the Pacific Northwest, and acaricides used to control the mites are often inadequate.

Development of resistant varieties has eased pest problems in many crops (Beck 1965). Some researchers have reported differences in mite susceptibility among hop varieties. Regev and Cone (1975b) reported that hop varieties differed in susceptibility to mites under both field and greenhouse conditions. Mayberry (1968) tested different hop genotypes for their effects on mite life histories under laboratory conditions. Berry (unpublished data) found differences in varietal susceptibility to twospotted spider mites in the greenhouse.

Seven commercially-grown hop varieties were compared to determine their relative susceptibility to mite populations. Population counts were taken on naturally infested plants in the field, and on artificially infested plants in the greenhouse. Fertility schedules were prepared for mite populations reared on different varieties under laboratory conditions.

Methods and Materials

Greenhouse Comparison

The greenhouse test was conducted in June, in a room with a mean temperature of 24°C and natural lighting conditions. Potted hop plants of five varieties were distributed in a completely random pattern on a greenhouse bench. The varieties used, and the number of plants per variety, were: L-1 (5), L-8 (4), Cascade (3), Comet (2), and Fuggle (3). Because some varieties are more densely leaved than others, the number of leaves on each plant was counted. Each plant was infested with adult female spider mites taken from a culture maintained on lima beans in the greenhouse, using an infestation level of one mite per three mature leaves.

Seventeen days after inoculation, five leaves were removed from each plant, and the number of immature and adult mites and eggs was counted.

Fertility Schedule

Leaves were collected from varieties L-16, Talisman, Comet, Cascade, and Fuggle grown at the USDA hop breeding facility near Corvallis, OR. Discs 1.56 cm^2 were cut from the leaves with a no. 7 cork borer and floated on a layer of vermiculite in a petri dish filled with distilled water. Each disc was observed under a

binocular microscope (20X) and any eggs or mites found were removed with a small paintbrush.

Quiescent female deutonymphs and adult males from a two-spotted spider mite culture maintained on lima bean plants were placed in a 1:1 ratio on leaf discs cut from the hop varieties. The following day, females which had ecdysed were placed singly on fresh leaf discs from the hop variety on which they had emerged.

The females were maintained in a controlled environment room held at 26°C with a photoperiod regime of 16 hours light:8 hours darkness until their deaths. Each female was moved to a fresh leaf disc three times a week, and each disc was saved and observed three times a week for the development of female progeny. Records were kept on the survival of the parent females, the number of female progeny produced, and the development time of the female progeny. A sample of female progeny from each variety was saved to obtain data on their adult lifespans.

Data from the fertility schedule experiment were analyzed using methods reported by Southwood (1968) and Isaacson.[2]

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[2]Isaacson, D. 1974. Documentation for *GROPAR (a biology program). Conduit Computer Center. Oregon State University, Corvallis.

Field Comparison

Hop plants of the varieties L-16, Talisman, Comet, Cascade, and Fuggle were sampled to determine spider mite density. The plants, grown at the USDA hop breeding facility, were arranged in unreplicated variety blocks. Eight mature leaves of medium size were picked from each of three plants per variety. Because Mayberry (1968) found that mite populations varied with their height on the plant, all leaves were picked from the area between ca. 1 and 2 m from the ground. Immature and adult mites (excluding eggs) were counted on each leaf.

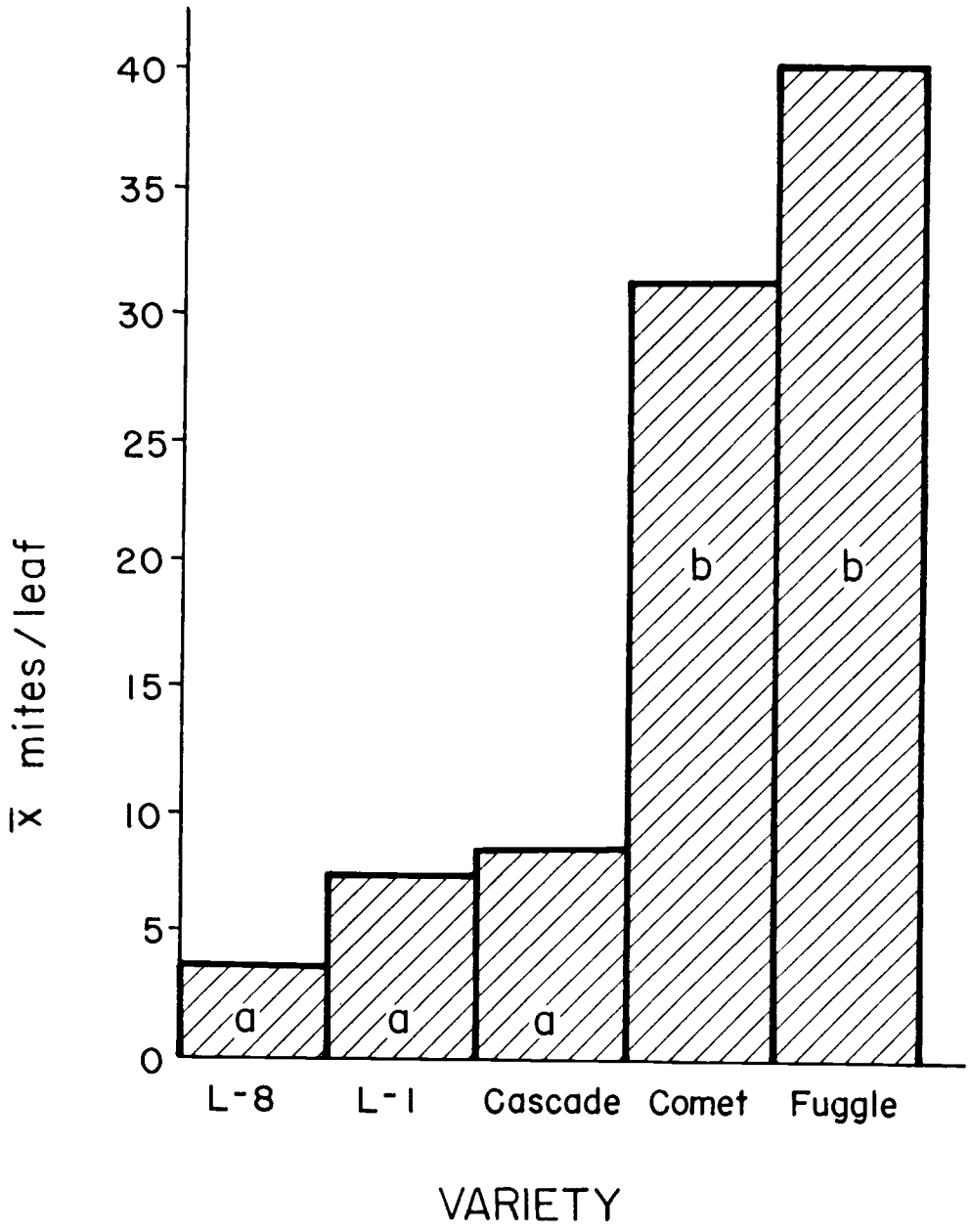
Results and Discussion

Greenhouse Comparison

Nested analysis of variance of the mite density on varieties grown in the greenhouse showed significant ($P = 0.05$) differences among varieties and among plants within varieties (Fig. 2). Comet and Fuggle developed the highest mite populations, with 39.93 and 31.51 mites/leaf, respectively. Using Duncan's New Multiple Range Test ($P = 0.05$), Comet and Fuggle were significantly different from L-8, L-1, and Cascade, which had means of 3.74, 7.17, and 8.18 mites/leaf, respectively. (Analysis of variance was performed on log-transformed data; means are back-transformed.)

Figure 2. Mean density of twospotted spider mites on leaves of five hop varieties grown in the greenhouse. Means with letters in common are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

Figure 2


40
35
30
25
20
15
10
5
0
x̄ mites / leaf
a
a
a
b
b
L-8
L-1
Cascade
Comet
Fuggle
VARIETY

These results are similar to those obtained in greenhouse tests by Berry (unpublished data), in which two late cluster varieties (L-1 and L-16) had significantly lower populations than Cascade, Comet, and Fuggle ($P \leq 0.05$).

Regev and Cone (1975b) reported that in field and greenhouse tests, Comet and Cascade were susceptible to twospotted spider mites, while L-1 and L-8 were resistant.

In all of these tests, Comet and Fuggle were classified as susceptible, while the late cluster varieties demonstrated resistance. Cascade develops populations of intermediate size.

Fertility Schedule

Results of the fertility schedule analysis are presented in Table 1. Southwood (1968) indicated that R_m, the intrinsic rate of natural increase, is the most valuable statistic for describing population growth. Mites on Cascade had the highest R_m value, followed in decreasing order by Talisman, Fuggle, Comet, and L-16. L, the finite rate of natural increase, is the number of times a population multiplies per time unit (per day). The varieties are arrayed in the same order for L as for R_m, because L is a positive function of R_m. R_O, the net reproductive rate, describes the number of times a population multiplies per generation. R_O was highest for mites on Fuggle, followed by those on Comet, Cascade, Talisman,

Table 1. Fertility schedule statistics for mites reared on five varieties of hops.

Variety	R_O	R_m	T	L
Cascade	500.87	0.268	23.115	1.309
Comet	558.74	0.255	24.841	1.290
Fuggle	599.46	0.258	24.830	1.294
Talisman	478.74	0.259	23.808	1.296
L-16	383.45	0.243	24.449	1.276

and L-16, respectively. The generation time, T, is the mean age of mothers at the appearance of female offspring. Generation time was lowest (most rapid) for mites on Cascade, followed in increasing order by those on Talisman, L-16, Fuggle, and Comet.

Interpretation of fertility schedule statistics is complicated by the fact that significance tests cannot be conducted on them, and because the methods are best suited to organisms with non-overlapping generations (Southwood 1968).

Field Comparison

Results of the field counts are shown in Table 2. Significant differences ($P \leq 0.005$) were found among varieties and among plants within varieties using nested analysis of variance. The differences among varieties were dissimilar to those found in the greenhouse test. Fuggle and Cascade had the lowest mite densities, Talisman and Comet had intermediate densities, and L-16 had the highest density.

Conclusion

The greenhouse comparison confirmed earlier reports that the late cluster varieties are resistant to twospotted spider mites, and that Fuggle and Comet are susceptible.

In the fertility schedule analysis, none of the varieties gave consistently high or low values for all of the parameters measured.

Table 2. Mean number of mites per leaf on five varieties of hops grown under field conditions.

Variety	$\bar{x} \pm$ SE mites/leaf[a]
Fuggle	0.75 ± 0.33 a[b]
Cascade	2.79 ± 1.14 a
Talisman	10.33 ± 2.35 b
Comet	10.58 ± 1.80 b
L-16	21.71 ± 4.75 c

[a]Average of five leaves from each of three plants.

[b]Means followed by the same letter are not significantly different (P = 0.05) using Duncan's New Multiple Range Test.

For example, Fuggle had the highest R_o value, but ranked third and fourth for R_m and T, respectively. However, differences among varieties for some of the statistics appeared to be large, and might indicate important aspects of resistance. Net reproductive rates ranged from 383.45 (L-16) to 549.46 (Fuggle), and the highest and lowest generation times differed by 1.73 days.

The results of the field comparison differed from the greenhouse results and from the reports of other researchers concerning relative susceptibility and resistance of hops to the twospotted spider mite. Experimental error may have contributed heavily to the field results. The plants sampled were distributed in variety blocks, and apparent differences among varieties may have been caused by clustering of the mite population within the field. Mayberry (1968) also reported difficulty in demonstrating varietal resistance in the field.

In both the greenhouse and field studies, mite densities differed significantly ($P \leq 0.05$) among plants of the same variety. Because a large degree of variation occurs within varieties, a large number of plants should be sampled if varietal characteristics are to be adequately measured.

IV. EFFECTS OF RESISTANT AND SUSCEPTIBLE HOP VARIETIES ON OVIPOSITION, DEVELOPMENT, SURVIVAL, AND SEX RATIO OF THE TWOSPOTTED SPIDER MITE

Introduction

Resistant plants may affect a pest by being a non-preferred host, by tolerating the pest's attack, or by exerting antibiotic effects against the pest (Painter 1951). The use of antibiosis by plants resistant to the twospotted spider mite (Tetranychus urticae Koch) has been noted by several researchers. Toxicity to the twospotted spider mite was reported by Patterson et al. (1974); decreased fecundity was noted by Patterson et al. (1974), Gentile (1969), and Schuster et al. (1972a, b); and lengthened developmental rates were found by Schuster et al. (1972b).

Mayberry (1968) tested many hop varieties and genotypes for resistance to the twospotted spider mite, using fecundity measurements to evaluate resistance. He also compared developmental rates of mites on some hop genotypes.

In this study, mites were reared on several commercial varieties of hops. Oviposition, sex ratio, developmental rates, and survival rates were compared, to determine which aspects of the twospotted spider mite's life history are affected by hop varieties. Hop varieties were used which have been noted for their

resistance or susceptibility in previous studies (Regev and Cone 1975b, Berry [unpublished data]).

Materials and Methods

Experiments were conducted in a controlled environment room with a 16 hour light:8 hour dark photoperiod, during 1976 and 1977. In both years, mites were obtained from cultures of the twospotted spider mite maintained on lima beans in the controlled environment room.

Fresh hop leaves were obtained in both years from plants at the USDA hop breeding facility near Corvallis, OR. Mature leaves which showed a minimum degree of arthropod infestation or damage were selected for use in the experiments. Discs cut from the leaves were examined under a binocular microscope (20X) and foreign material, including mites and mite eggs, was removed with a small camel's-hair brush.

1976 Study

Experiments on oviposition, survival, developmental rates, and sex ratio of mites reared on the hop varieties Fuggle, Cascade, L-16, and L-1 were conducted during August and September of 1976. The controlled environment room was held at 23.9^{o}C during this period.

Hop leaves and mites were held in a modified Munger cell apparatus (Munger 1942) developed by Berry (unpublished). The apparatus (Fig. 3) consists of a 20.3 cm x 25.4 cm plywood board covered with a sheet of blotting paper and two 3 mm thick Plexiglas(R) sheets cut to the same dimensions as the board. Twenty-four holes 3.5 cm in diameter were cut in one plexiglas sheet, which was laid on top of the blotting paper. Hop leaf discs 4 cm in diameter were placed ventral side up beneath the holes, and the blotter paper was moistened with distilled water to keep the leaves moist. The second sheet of plexiglas was placed on top of the first, and bolts were run through the plywood base and plexiglas sheets and secured with wing nuts. The bolts pressed the plywood and plexiglas sheets together and discouraged migration of mites among the cells.

Quiescent female deutonymphs were isolated on leaf discs of the four hop varieties, and two adult males were provided for each female to insure mating. One female was placed on each disc. After the females ecdysed, they were transferred daily to fresh leaf discs of the same variety, and their daily egg production was recorded. The leaf discs were kept in the chambers and observed at two to three day intervals. Records were kept on the number of progeny that died or disappeared before attaining maturity, and on the number and sex of progeny which reached the adult stage. Eggs

Figure 3. Modified Munger apparatus.

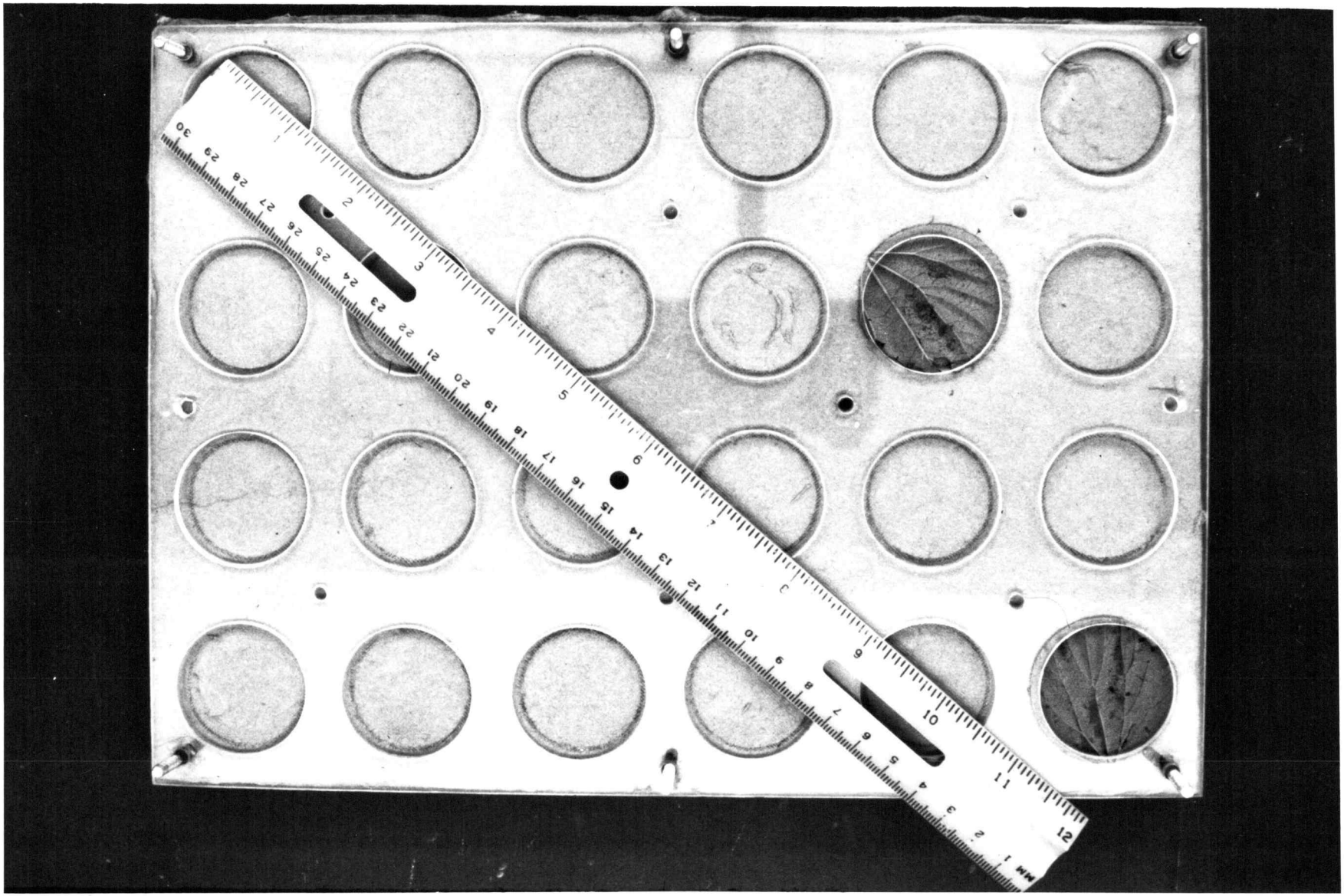


Fig. 3

laid during the first five days of each female's oviposition period were observed daily to obtain data on developmental rates.

This experiment was also designed to evaluate the effect of maintaining several generations of mites on one hop variety. As soon as adult females appeared on the leaf discs, they were transferred to leaf discs of the same variety to start a second generation of mites. Females were chosen which were less than one day old, and had been on leaf discs with at least two adult males. One female was selected from the progeny produced by each female in the original experiment. When the female progeny of the second generation reached maturity, a third generation of mites was established on leaf discs of the same variety using the same procedures as before.

Five adult females were used on each variety in the first generation. Less than five parent females were used on some varieties in the second and third generations, because too few females were produced which met the criteria for starting a new generation.

1977 Study

Further experiments on oviposition, mortality, developmental rates, and sex ratio were conducted in 1977 using different laboratory techniques. The temperature of the controlled

environment room was increased to 25.6°C, and the experiments were conducted in June, July, and August. Instead of using the modified Munger cells, leaf discs 1.25 cm in diameter (1.56 cm^2) were floated on distilled water in petri dishes partially filled with vermiculite.

Two experiments were conducted to investigate the effects of the different hop varieties on oviposition. In the first experiment, adult female mites were held during their entire lives on leaf discs cut from Cascade, Comet, Fuggle, Talisman, and L-16. The number of eggs produced by each female was counted three times weekly, until all of the females died. Sixteen females were used on all varieties except Comet, which had 17 females.

In the second oviposition experiment, a nested design was used to measure variation among mites held on the same variety. Leaves were collected from four varieties: Cascade, Talisman, L-16, and Comet. For each variety, three plants were sampled. Three leaves were taken from each plant, and three discs were cut from each leaf. A six-day-old virgin female mite was confined on each disc for five days, after which the eggs on each disc were counted. A total of 27 mites was tested on each variety.

An experiment was also conducted to measure differences in survival, development time, and sex ratio on the varieties Talisman, Comet, Fuggle, Cascade, and L-16. Fourteen leaf discs

were cut from each variety, and four mated females were confined on each disc for one day. The females were removed, the eggs were counted, and each disc was marked for identification. The discs were observed daily to determine the number of eggs which survived to maturity, the number of adult males and females produced, and their developmental rate.

Results

1976 Study

During the first generation, significant differences were found in oviposition ($P \leq 0.005$) and sex ratio ($P \leq 0.025$). These factors were not significantly different in the second and third generations ($P \geq 0.05$). Developmental rates differed significantly ($P \leq 0.005$) during both the first and second generations, but not in the third. Survival rates differed significantly only during the second generation ($P \leq 0.05$).

Results of these experiments are shown in Tables 3-6. Mites reared on lima beans and then maintained on varieties L-1 and Fuggle produced significantly more eggs than those reared on L-16 and Cascade (Table 3). The first generation of progeny produced on Cascade had a significantly smaller proportion of females than the first generation progeny produced on the other three varieties (Table 5). First generation progeny developed more

Table 3. Mean number of eggs produced per day by three generations of mites on four hop varieties.

Variety	$\bar{x} \pm$ SE eggs/day		
	Generation I	Generation II	Generation III
L-1	8.14 ± 0.45 a[a]	8.20 ± 1.60	5.71 ± 1.04
Fuggle	7.80 + 0.83 a	10.51 + 0.80	—[b]
L-16	4.04 ± 1.43 b	9.56 ± 0.71	6.01 ± 0.55
Cascade	3.70 ± 0.51 b	8.56 ± 0.66	5.57 ± 0.45

[a]Means followed by the same letter are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

[b]Missing data.

Table 4. Survival of immature mites on four hop varieties. Mean percentage of eggs surviving to the adult stage.

Variety	$\bar{x} \pm$ SE % survival		
	Generation I	Generation II	Generation III
Cascade	51.29 ± 13.36	45.10 ± 9.90 a[a]	26.13 ± 7.86
L-16	59.20 ± 16.59	43.73 ± 5.99 a	17.25 ± 10.87
L-1	60.68 ± 10.59	41.03 ± 6.52 a	8.95 ± 6.77
Fuggle	62.65 + 6.85	12.02 + 7.00 b	—[b]

[a]Means followed by the same letters are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

[b]Missing data.

Table 5. Sex ratio of mites on four hop varieties. Mean percentage of progeny which were female.

Variety	$\bar{x} \pm$ SE % female progeny		
	Generation I	Generation II	Generation III
L-16	84.33 ± 5.24 a[a]	79.01 ± 4.49	58.18 ± 21.41
L-1	79.90 + 2.14 a	72.40 + 8.35	98.10 + 1.90
Fuggle	79.58 + 5.45 a	70.56 + 1.33	—[b]
Cascade	64.29 ± 3.61 b	60.60 ± 8.07	73.13 ± 9.21

[a]Means followed by the same letter are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

[b]Missing data.

Table 6. Mean developmental time from egg to adult for three generations of mites reared on four hop varieties.

Variety	$\bar{x} \pm$ SE development time (days)		
	Generation I	Generation II	Generation III
Fuggle	11.32 ± 0.16 a[a]	11.03 ± 0.16 a, b	10.50 ± 0.29
L-1	11.57 ± 0.18 a	10.02 ± 0.61 b	10.79 ± 0.36
L-16	12.58 ± 0.27 b	11.94 ± 0.20 a	10.55 ± 0.41
Cascade	13.11 ± 0.33 b	12.49 ± 0.24 a	9.93 ± 0.29

[a]Means in the same column followed by the same letter are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

rapidly on Fuggle and L-1 than on L-16 and Cascade (Table 6). In the second generation, significantly fewer eggs survived to maturity on Fuggle than on the other three varieties (Table 4), and developmental rates varied significantly (Table 6). No significant differences occurred in the third generation among the four varieties tested.

1977 Study

In the first oviposition experiment, females had stopped producing eggs by the 38th day of oviposition. Sixteen egg counts were conducted during this period, at two- or three-day intervals. Analysis of variance showed significant differences ($P \leq 0.05$) among varieties during only three of the intervals; results of these analyses are shown in Table 7.

At the end of the oviposition period, the females had produced an average of 227.68, 227.53, 227.51, 225.26, and 208.77 eggs on Comet, Cascade, L-16, Fuggle, and Talisman, respectively (the means were obtained by summing the mean number of eggs laid on each variety during each counting interval).

In the second oviposition experiment, the number of eggs laid did not vary significantly among varieties ($P \geq 0.05$). The results are shown in Table 8. Nested analysis of variance showed that variation among mites on leaves of the same plant was not

Table 7. Mean number of eggs laid by female mites on leaf discs of five hop varieties, during periods when means were significantly different (P = 0.05).

Variety	$\bar{x} \pm$ SE eggs/leaf disc[a] on indicated oviposition day		
	Day 3[b]	Day 7[c]	Day 12[d]
Cascade	10.89 ± 1.73 ab[e]	26.56 ± 0.58 a	21.56 ± 0.77 ab
Comet	15.44 ± 1.26 a	25.67 ± 0.55 a	19.11 ± 0.73 b
Fuggle	8.89 ± 1.29 b	23.44 ± 1.42 b	22.78 ± 0.98 a
Talisman	12.00 ± 0.96 ab	22.56 ± 1.02 b	20.11 ± 0.72 b
L-16	12.33 ± 2.03 ab	24.22 ± 0.66 ab	19.89 ± 1.09 b

[a]Average of nine females per variety.

[b]Eggs had accumulated on the leaf discs for three days.

[c]Eggs had accumulated on the leaf discs for seven days.

[d]Eggs had accumulated on the leaf discs for five days.

[e]Means in the same column followed by the same letter are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

Table 8. Mean number of eggs laid on leaf discs of four hop varieties by unmated female two-spotted spider mites during a period of five days.

Variety	$\bar{x} \pm$ SE Eggs/leaf disc
Cascade	36.56 ± 0.93
Talisman	36.28 ± 0.80
L-16	34.39 ± 0.68
Comet	34.06 ± 0.86

Table 9. Mean development time of twospotted spider mites reared on five varieties of hop. Mean represents days elapsed from deposition of the egg to emergence of the adult from the last molt.

Variety	$\bar{x} \pm$ SE Development time (days)	n
Talisman	12.72 ± 0.10 a[a]	203
L-16	12.35 ± 0.06 b	310
Comet	12.04 ± 0.05 c	394
Cascade	11.80 ± 0.04 d	299
Fuggle	11.73 ± 0.06 d	308

[a]Means followed by the same letter are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

significant, but variation among plants of the same variety was significant ($P \leq 0.025$).

Developmental rates of mites reared on different varieties varied significantly ($P \leq 0.005$). The time from deposition of the eggs to appearance of adults varied by slightly less than one day, with mites on Talisman having the slowest rate and mites on Fuggle and Cascade having the highest rates (Table 9).

Survival did not vary significantly ($P \geq 0.05$). On L-16, 71.93% of the eggs laid survived to adulthood. Percent survival on Cascade, Fuggle, Comet, and Talisman were 82.11%, 83.11%, 86.45%, and 86.60%, respectively.

Differences in the sex ratio of progeny were also not significantly different among the varieties. The percentages of adult progeny which were female were 67.95%, 65.55%, 61.63%, 60.54%, and 54.81% for mites on Cascade, Talisman, Fuggle, Comet, and L-16, respectively.

Discussion

Experimental difficulties encountered during the 1976 experiment were largely eliminated by changes in experimental technique in 1977. In 1976, leaves in the field began senescence in late August and September, and many leaves were heavily infested by aphids and mites. In 1977, experiments were terminated in August

to avoid this problem. In 1976, leaves of poor quality were attacked by fungal growths when held in the modified Munger cells, especially during the later stages of the experiment. The fungus clearly affected developmental rates and mite survival. Fungal growths were almost eliminated in 1977 by holding leaf discs in petri dishes, by using better quality leaves, and by raising the temperature of the controlled environment room to increase the developmental rate of the mites, which reduced the time that individual leaf discs had to be held. Changing the size of the leaf discs from 16 cm^2 to 1.56 cm^2 helped to reduce counting errors, and facilitated the use of more mites in each experiment.

Experimental error interfered with observations on generation effects in the 1976 test. Fungal attacks were not severe in the first generation, but in the second and third generations they became increasingly acute. Differences in survival and developmental times in the second generation were probably markedly influenced by fungal growths. Because of this problem, discussion on the 1976 test will be limited to the first generation.

Differences in oviposition occurred in the first generation in the 1976 test, but the two experiments conducted in 1977 failed to confirm these findings. Of 16 egg counts conducted in the first 1977 experiment, only three showed significant differences among varieties, and in the significant tests none of the varieties had a

consistent effect on egg production. The total number of eggs produced by females on the five varieties was quite close. Since the total egg production was calculated by summing a series of means, it does not account for possible differences in adult female survival, which could be an important factor in determining egg production by mite populations. The second 1977 oviposition test showed no significant differences among varieties, but did indicate that oviposition on different plants of the same variety varied significantly. This suggests that if varieties do influence oviposition, large numbers of plants should be sampled if varietal effects are to be adequately characterized.

Survival rates differed significantly only during the second generation of the 1976 experiment, when fungal attacks on the leaf discs were identified as an interfering factor. It appears that none of the varieties had a significant effect on survival of immature mites. Survival of adult mites was not measured.

Significant differences occurred in the sex ratio of mites maintained on different varieties in the first generation of the 1976 experiment. In the 1977 study, differences in the sex ratio were significant at the 10%, but not at the 5%, level of probability. Since female mites are arrhenotokous, it is possible that the host plant influences the fertilization efficiency of the mites. Sex ratio could also be affected by differences in survival of immature males and

females, or by differences in migration of males and females out of the Munger cells.

Significant differences occurred in the development rate of mites reared on different varieties. In both years, highly significant ($P \leq 0.005$) differences were observed, and in both years mites on Fuggle developed rapidly while mites on L-16 developed slowly. Development on Cascade was inconsistent; the rate on Cascade was relatively slow in 1976 but was rapid in 1977. In spite of this inconsistency, these experiments offer good evidence of a varietal effect on immature development. An increase in development time could deter the build-up of mite populations over a period of several generations.

In 1977, mites reared on Talisman and L-16 had a different appearance than mites maintained on other varieties. In their immature stages, mites on Talisman and L-16 developed a spotty distribution of color, giving a granular effect when viewed dorsally. Mites on Cascade, Comet, and Fuggle developed the large, dense spots typically associated with the twospotted spider mite. No differences were noted after the adult stage was reached. Differences in color distribution might be related to feeding frequency or to the quantity of food ingested.

V. LEAF CHARACTERISTICS OF SELECTED HOP VARIETIES, AND THEIR RELATIONSHIP TO MITE OVIPOSITION, SURVIVAL, DEVELOPMENT, AND SEX RATIO

Introduction

The twospotted spider mite (Tetranychus urticae Koch) is distributed throughout the world and attacks many crops. The literature indicates that in several crops, cultivars and breeding lines differ in their susceptibility to mites, with some plants displaying resistant traits.

Several researchers have reported that morphological differences among varieties are related to resistance to the twospotted spider mite. Glandular hairs have been associated with resistance in tobacco (Patterson et al. 1974) and tomatoes (Rodriguez et al. 1972), and foliar pubescence has been related to resistance in strawberries (Kishaba et al. 1972). The presence of glands on cotton cultivars was related to mite resistance by Schuster et al. (1972).

In this study, hop leaves from different varieties were compared to determine what morphological differences occurred, and the relationship of the morphological features to mite oviposition, survival, developmental rate, and sex ratio were examined. The morphological characteristics studied were ventral hairs and glands,

which have been reported to be more dense on some varieties than others (Burgess 1964). In addition, the moisture content of leaves from different varieties was measured, because field observations indicated that some varieties were more succulent than others. Leaf area was also measured and related to mite life histories.

Methods and Materials

All leaves used in this study were collected from hops growing at the USDA hop research facility near Corvallis, OR. Leaves were selected which showed minimum evidence of arthropod infestations or senility.

Discs cut from the leaves were immediately floated ventral side up on distilled water, in petri dishes partially filled with vermiculite. The floating vermiculite formed a flat surface which aided observation of the leaf discs under a dissecting microscope. The dishes were refilled with distilled water daily.

All leaf discs which were to be infested with mites were examined under a binocular microscope (20X) and all debris, including mites and eggs, was removed using a fine camel's-hair brush.

Mites used to infest the leaf discs were obtained from a culture maintained on lima beans. All experiments were conducted in a constant temperature room maintained at 26^{o}C, with a photoperiod regime of 16 hours light:8 hours darkness. The mite culture was kept under the same conditions.

Varietal Characteristics

A preliminary study was conducted to determine the degree of variation among and within varieties in the density of ventral glands and hairs on hop leaves and in the moisture content of the leaves.

Leaves were collected from the varieties Cascade, Comet, L-16, and Talisman. Three plants of each variety were sampled, and three leaves were removed from each plant. Immediately after collection, three 1.56 cm^2 discs were cut from each leaf using a No. 7 cork borer. The remaining portions of the leaves were immediately weighed with a Metler® balance, and placed in a 70°C drying oven. Two days later, the dried leaves were removed and reweighed to obtain a ratio of dry to wet weight ("weight ratio").

Each disc was observed under a binocular microscope and the number of ventral glands and hairs was counted (Fig. 4). Glands were counted under 20X magnification and hairs under 40X magnification.

Effect of Varietal Characteristics on Mite Life History

Mites were reared on leaves from five hop varieties. The mites' oviposition, sex ratio, development rate and survival rate were related to the varietal characteristics of the leaves they were reared on, using linear regression.

Figure 4. Ventral surface of a Cascade leaf, showing glands (A) and vein hairs (B).

Fig. 4

Leaves were collected from Cascade, Comet, Fuggle, Talisman, and L-16. Three plants were sampled from each variety, and five leaves were removed from each plant. A 1.21 cm^2 disc was cut from each leaf using a No. 6 cork borer. The leaf discs were floated on distilled water and infested with one mated, five-day-old female mite per disc. The females were removed one day later, and the eggs laid on each disc were counted. The discs were observed daily to obtain data on developmental rates, survival, and sex ratio. As adults appeared, they were counted, sexed, and removed.

Ventral glands were counted on each disc, and the dry:wet weight ratio was determined for each leaf, as previously described.

Ventral hairs were counted using different methods than those described previously. In the previous experiment, all the hairs on each leaf disc were counted, but it was apparent that more hairs were found on the surface of the veins than in interveinal areas. In addition, the density of hairs on the veins appeared to be positively correlated with the size of the veins. Since mites usually concentrate in areas bounded by the larger veins, it is likely that they would have more contact with hairs found on the leaf surface and the small veins than with hairs on the large veins. Accordingly, the hairs were counted separately in this experiment. Hairs growing on the leaf surface and the minor veins were termed "leaf hairs," while those

growing on the major veins were termed "vein hairs." The major veins were identified as those which were clearly differentiated from the surrounding leaf tissue by their color and surface texture. All of the leaf hairs on each disc were counted. On each disc, a 5 mm length of one major vein was examined for vein hairs.

In the previous experiment, it was apparent that the size of the leaves varied widely on each plant sampled, and it appeared that large leaves were usually older, and in poorer condition, than small leaves. In this experiment, the area of each leaf was measured using a Li-Cor® Portable Area Meter before the leaf discs were cut.

Effect of Varietal Characteristics on Mite Migration

In a preliminary experiment, mites were allowed to migrate among leaf discs of different hop varieties to determine whether they displayed a preference for some varieties. Leaf discs 16 cm^2 in area were cut from hop leaves of the varieties L-16, Cascade, Fuggle, Talisman, and Comet. Four discs of each variety were used. The discs were placed in a completely random arrangement in a modified Munger cell apparatus (described in Chapter III). The top cover of the apparatus was removed to allow free migration of mites among the discs. Vials (25 ml) partially filled with distilled water were inverted over the empty cells to keep the blotter paper of the

apparatus damp. Each disc was infested with 12 adult female mites. Three days later, the mites on each disc were counted.

In a second experiment, three leaves were taken from each of the varieties used in the preliminary experiment. The area of each leaf was measured, and after cutting a 16 cm^2 disc from each leaf, the remains of the leaves were weighed and dried. The leaf discs were distributed in a completely random arrangement in the modified Munger cell apparatus (with the top cover removed), and each disc was infested with 20 female mites. One day later, the mites on each disc were counted. A 1.21 cm^2 disc was cut from each 16 cm^2 disc and used for gland and hair counts, using the methods described in the previous section.

Results

Varietal Characteristics

Highly significant differences ($P \leq 0.005$) were found among varieties for all of the characteristics studied. However, nested analysis of variance indicated that considerable variation in these characteristics also occurred within varieties.

Table 10 shows the mean weight ratios for each variety. Cascade and Comet were the most moist varieties, and were not significantly different from each other using Duncan's New Multiple Range Test ($P \geq 0.05$). L-16 and Talisman were significantly

Table 10. Mean ratio of dry:wet weights in leaves of four hop varieties (expressed as a percentage).

Variety	Percent dry weight ± SE[a]
Talisman	27.96 ± 0.92 a[b]
L-16	25.69 ± 0.86 b
Comet	22.49 ± 0.63 c
Cascade	22.00 ± 0.69 c

[a]Average of nine leaves from each variety.

[b]Means followed by the same letter are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

different from each other and from Cascade and Comet. Differences among plants within varieties were not significant ($P \geq 0.05$).

Gland densities are presented in Figure 5. Comet had the highest density of glands, followed in decreasing order by Cascade, L-16, and Talisman. Differences among plants within varieties were not significant, but variation among leaves within plants was highly significant ($P \leq 0.005$).

Hair density (Fig. 6) varied widely among varieties. L-16 had the greatest density of hairs, followed in decreasing order by Talisman, Cascade, and Comet. Significant differences also occurred among plants within varieties ($P \leq 0.05$) and among leaves within plants ($P \leq 0.01$).

Effect of Varietal Characteristics on Mite Life History

Data from this experiment were analyzed using linear regression. The measurements of varietal characteristics (leaf area, weight ratio, leaf hair density, vein hair density, and gland density) were used as independent variables, and the mite data were used as dependent variables. Five dependent variables were analyzed: oviposition (the number of eggs laid by a single female in one day); survival (the proportion of eggs laid on a disc which survived to adulthood); development rate (the mean time from egg to adult for progeny of a single female); female development rate (the mean time

Figure 5. Mean density of ventral glands on hop leaves of four varieties. Means with letters in common are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

Figure 6. Mean density of ventral hairs on leaves of four hop varieties. Means with letters in common are not significantly different using Duncan's New Multiple Range Test (P = 0.05).

Figure 5

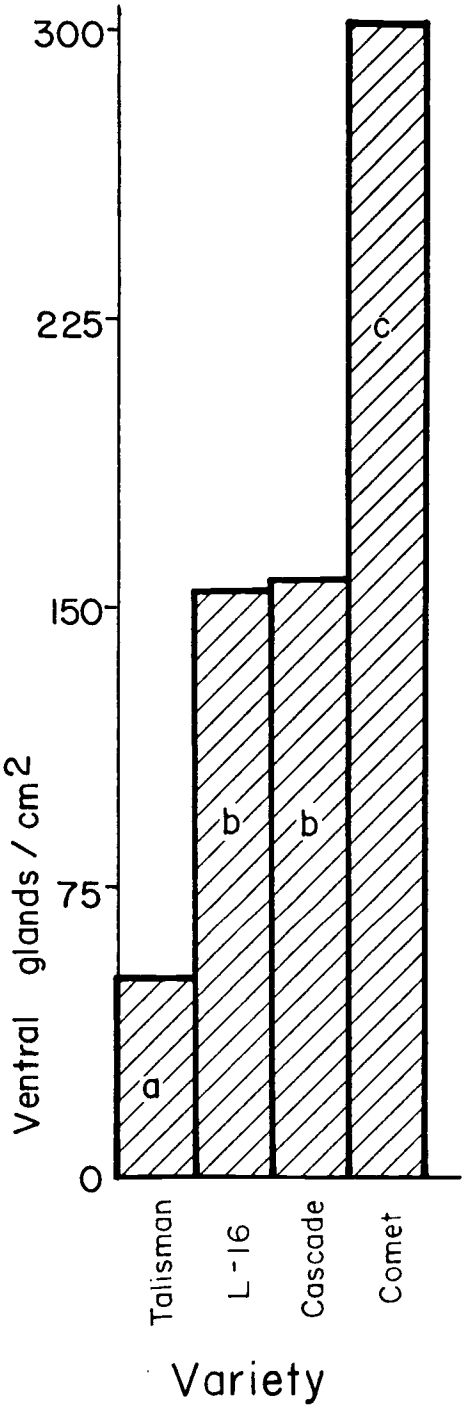
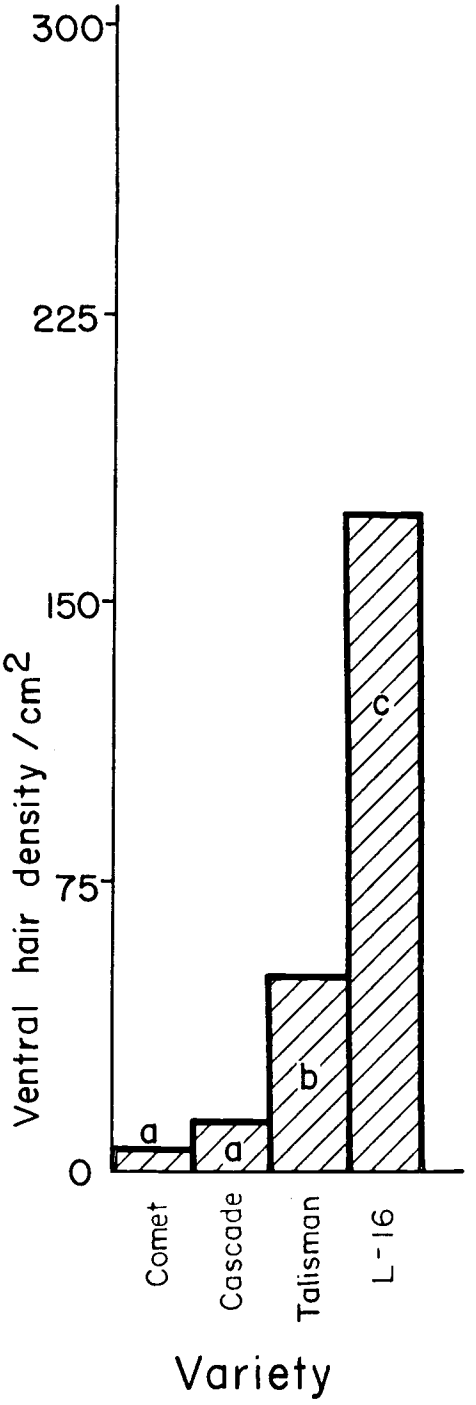


Figure 6



from egg to adult for the female progeny of a single female); and "female ratio" (the number of adult female progeny produced by a female divided by the total number of adult progeny).

Two sets of regressions were conducted. In the first set, the data from all varieties were pooled and each dependent variable was regressed with each independent variable. Analysis of variance was conducted on each regression, and any that were significant ($P \leq 0.05$) were included in the second set of regressions, in which the data from different varieties were separated.

This discussion involves only simple linear regressions which were significant according to analysis of variance ($P \leq 0.05$). Non-significant simple regressions are reported in Appendix I. A series of multiple regressions conducted on the pooled data is reported in Appendix II.

The simple linear regressions which were significant are reported in Table 11, along with the coefficient of determination, which designates the amount of variation in the dependent variable attributable to variation in the independent variable (Sokal and Rohlf (1969). Also noted is the slope of the regression line, which indicates whether the variables are related negatively or positively.

Oviposition - The pooled data and the data from Comet indicate that oviposition increased as leaf area increased. However, on

Table 11. Linear regressions relating mite reproduction, development, and sex ratio to hop leaf characteristics.

Dependent Variable (y)	Independent Variable (x)	Variety	Coefficient of Determination (R^2 x 100)		Slope of Regression Line
Oviposition	Leaf area	Pooled data[a]	10.23	(1, 61)[b]	+
		Talisman	46.57	(1, 8)	-
Female ratio[c]	Leaf hairs	Pooled data	10.45	(1, 60)	+
	Vein hairs	Pooled data	6.61	(1, 60)	+
		Talisman	49.06	(1, 8)	+
Development time[d]	Leaf weight ratio[e]	Pooled data	7.28	(1, 60)	+
		Talisman	45.92	(1, 8)	+
Development time	Leaf hairs	Pooled data	10.67	(1, 60)	+
		Talisman	57.57	(1, 8)	+
		L-16	32.85	(1, 12)	+
♀ Development time[f]	Leaf weight ratio	Pooled data	6.76	(1, 59)	+
		Talisman	52.36	(1, 8)	+

[a] Data from the five varieties were combined.

[b] Degrees of freedom

[c] Female ratio = female progeny produced/total progeny produced.

[d] Development time (days) from egg to adult for all progeny.

[e] Leaf weight ratio = dry weight of leaf/wet weight of leaf.

[f] Development time (days) from egg to adult for female progeny.

Talisman leaves, oviposition decreased as leaf area increased (Fig. 7).

Female Ratio - According to the pooled data analysis, the ratio of female progeny to total progeny increased as the number of leaf hairs increased, but this relationship was not significant in any of the varieties analyzed. Both the pooled data and the data from Talisman (Fig. 8) indicate a positive relationship between the female ratio and the number of vein hairs on the leaf.

Development Time - The development time (of male and female progeny combined) increased as the weight ratio increased, according to the analysis of the pooled data and of the Talisman data (Fig. 9). The development time was also positively related to the density of leaf hairs in analysis of pooled data, Talisman (Fig. 10), and L-16.

Female Development Time - The development time for female progeny increased as the weight ratio increased in regressions of the pooled data and of Talisman data (Fig. 11).

Survival - Regressions of survival against the leaf characteristics were conducted only on the pooled data; none of the regressions were significant.

Figure 7. Linear regression of oviposition and leaf area measurements from Talisman variety. $R^2 = 0.4657$. Eggs = 14.09 - 0.13 leaf area.

Figure 8. Linear regression of female ratio (female progeny/total progeny) and the density of ventral vein hairs for Talisman variety. $R^2 = 0.4906$. Female ratio = 0.01 + 0.04 vein hairs.

Figure 9. Linear regression of progeny development time (male and female progeny combined) and leaf weight ratios (dry weight/wet weight) for Talisman variety. $R^2 = 0.4592$. Development time = 7.19 + 15.70 weight ratio.

FIGURE 7

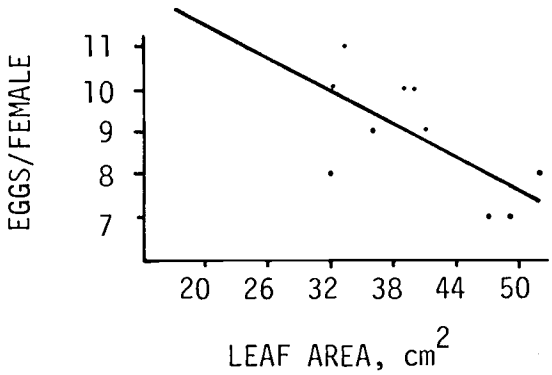


FIGURE 8

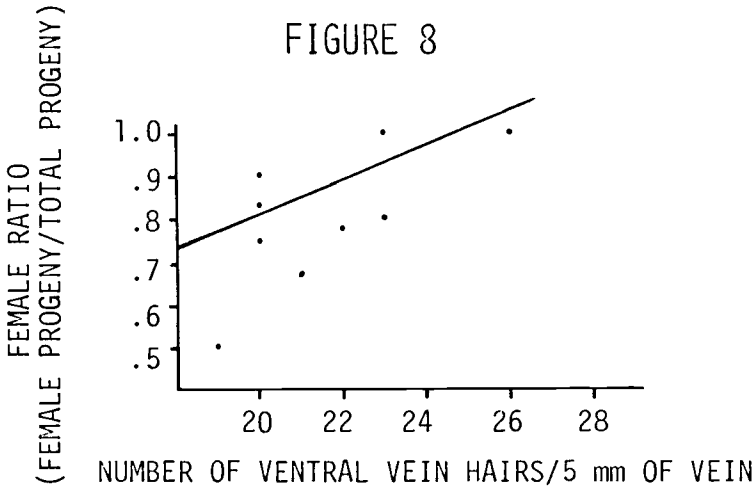


FIGURE 9

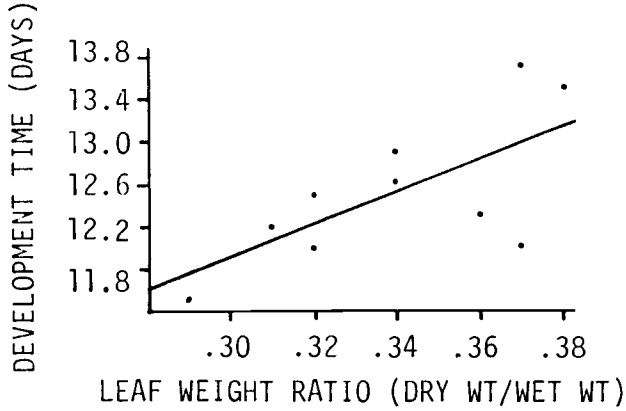


Figure 10. Linear regression of progeny development time (male and female progeny combined) and density of ventral leaf hairs for Talisman variety. $R^2 = 0.5757$. Development time = 11.81 + 0.04 leaf hairs.

Figure 11. Linear regression of female progeny development time (female progeny only) and leaf weight ratio (dry weight/wet weight) for Talisman variety. $R^2 = 0.5236$. Female development time = 7.23 + 15.93 weight ratio.

FIGURE 10

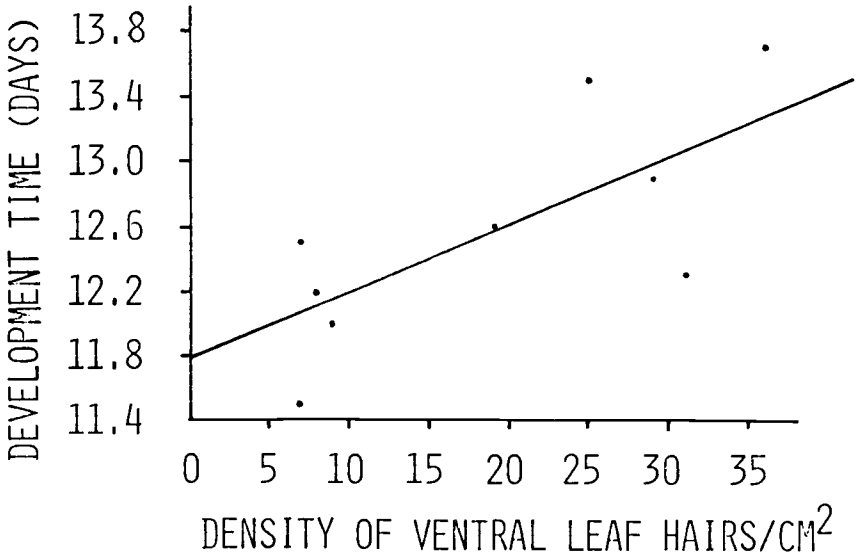
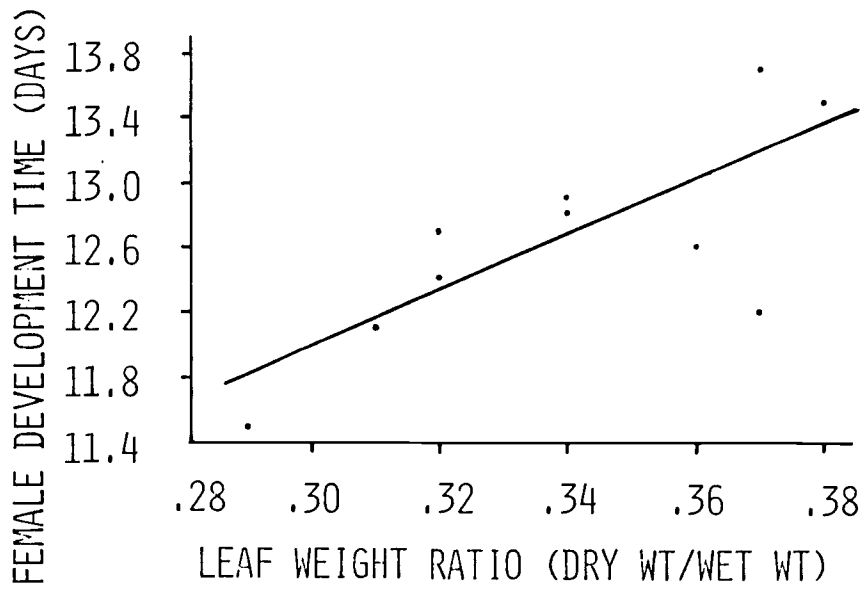


FIGURE 11



Effect of Varietal Characteristics on Mite Migration

The first migration experiment was analyzed using analysis of variance to determine whether differences occurred in female migration among varieties. The results were significant ($P \leq 0.01$). After migration an average of 3.5 mites were found on L-16 leaf discs, 4.8 on Fuggle and Cascade, and 5.0 on Talisman. All four varieties were significantly different from Comet, which had 22.8 mites per disc, using Duncan's New Multiple Range Test ($P = 0.05$).

In the second experiment, the number of mites found on each disc was used as the dependent variable in regressions on the data obtained from measurements of leaf characteristics (leaf area, leaf hair density, vein hair density, leaf weight ratio, and gland density). None of the simple regressions were significant. The results from simple and multiple regressions of the migration data are given in Appendices III and IV, respectively.

Discussion

The varieties studied differed widely in the density of ventral glands and hairs, and in the moisture content of the leaves. Because significant variation occurred among leaves of the same plant (gland and hair densities) and among plants of the same variety (hair density), large numbers of plants and leaves should be sampled if varietal characteristics are to be adequately characterized.

The dry:wet weight ratios were very consistent for plants of the same variety. This consistency may be due in part to differences in soil moisture, since the plants sampled were planted in variety blocks.

The regressions should be interpreted cautiously, since some of the R^2 values were quite low, and they do not constitute proof of association between the regressed variables. However, some of the results indicate that further investigation would be worthwhile. The regressions of data from Talisman have relatively high coefficients of determination, and the data coordinates appear to agree closely with the regression lines. Caution is also indicated in interpretation of the Talisman results, because only ten data points were used in the regressions.

Comet was preferred over the other varieties studied in the first migration test, but the following experiment showed no relationship between migratory preference and leaf characteristics.

VI. DETECTION OF FARNESOL IN HOP FOLIAGE, AND THE EFFECT OF FARNESOL ON FEMALE TWOSPOTTED SPIDER MITES

Introduction

Several terpenoid compounds mimic insect juvenile hormone (JH), including farnesol and farnesal (Bates et al. 1962), farnesyl methyl ether (Nair 1974), and geraniol (Hintze-Podufal 1975). JH influences insect metamorphosis, larval diapause, sex pheromone production, development of the ovaries, and deposition of yolk in insect eggs (Bowers 1976, Chapman 1969).

Terpenoids also act as insect sex pheromones. 2, 3-Dihydro-6, trans-farnesol is the principle component in the marking secretion of Bombus jonellus K. (Bergstrom and Svensson 1973), and (E)-B-farnesene is an alarm pheromone in several aphid species (Nishino et al. 1976).

Plants produce both JH mimics and sex pheromone mimics (Slama 1969, Williams 1970, Southwood 1973, Beck and Reese 1976). Several authors have discussed the possible effects of these compounds on insect populations (Clayton 1970, Beck and Reese 1976, Swain 1976).

Regev and Cone (1975a, b; 1976a, b) reported that terpenoid products of hops (Humulus lupulus L.) influence populations of the twospotted spider mite (Tetranychus urticae Koch). They identified

farnesol, nerolidol, and geraniol in ether extracts of pharate female twospotted spider mites (1975a, b). They reported that petroleum ether extracts of hop leaves contained farnesol and nerolidol, that foliar farnesol concentrations varied among hop varieties, and that varieties rich in farnesol developed larger mite populations than low farnesol varieties (1975b). Regev and Cone (1975a) found that male twospotted spider mites guarded powder clumps impregnated with farnesol, and in a later article (1976a) they reported that nerolidiol also stimulated guarding behavior. Topical applications of farnesol to pharate and adult females increased oviposition (1976b). They hypothesized that a dietary source of farnesol could favor mite populations by providing mites with a nutrient, a gonadotrophic hormone, or a sex attractant.

The purpose of this study was to develop a rapid, quantitative means of analyzing the farnesol content of hop foliage; to confirm Regev and Cone's reports that farnesol is related to hop resistance to the twospotted spider mite; to examine the variation of farnesol concentrations among hop varieties; and to relate foliar farnesol levels to the development of mite populations.

Methods and Materials

Preparation of Hop Foliage Extracts

The hop foliage examined in this study was obtained from the

USDA hop research facility near Corvallis, OR in September, 1976, and frozen in plastic bags until analysis.

All hop extracts were prepared using petroleum ether (30-60°C). Initially, the extracts were prepared from foliage which had been dried and ground to a powder with a mortar and pestle. The powder was swirled in petroleum ether, and the material was filtered through a funnel plugged with glass wool. The filtrate was concentrated under vacuum until the rate of evaporation was markedly slowed.

This method is similar to that reported by Regev and Cone (1975b). They dried their foliage in a 130°C oven. In this study, samples were initially dried at 180°C. However, a check on this method showed that when farnesol was added to powdered foliage and heated for one hour at 180°C, more than 80% of the farnesol was lost. After finding that farnesol was lost during exposure to high temperatures, the plant samples were dried at lower temperatures (120, 62, and 49°C, and room temperature).

Concern over the possibility of losing farnesol during the drying process led to the development of a wet extraction method. Frozen leaves were mixed in a blender with petroleum ether and anhydrous sodium sulfate. The blended material was filtered by suction in a Buchner funnel, and the filtrate was concentrated under vacuum until evaporation was markedly slowed. This usually

resulted in a final concentration of ca. 0.5 g (dry weight equivalent) foliage/ml petroleum ether.

Foliage samples from several varieties of hops were prepared using both the wet and dry extraction methods, and a sample of mature Fuggle H cones was prepared using the dry extraction method.

Thin Layer Chromatography Methods

All thin layer chromatography (TLC) plates were prepared with silica gel G, spread to a thickness of 250 μm.

One-Dimensional TLC Method

Crude ether extracts of hop foliage were spotted on TLC plates along with farnesol standards of known quantity (usually ranging between 15 and 25 μg). The plates were migrated 15 cm in a petroleum ether (30-60°C)-diethyl ether (PE:DE) (2:1) solvent system.

Two methods of farnesol detection were used. In the first, the plates were allowed to dry and then sprayed with a 1% solution of iodine in methanol. Iodine stains unsaturated compounds yellow. In the second method, the dried plates were sprayed with a 5% H_2SO_4 solution, and heated in a 180°C oven for ca. 5-10 min. This procedure singes all organic materials on the plate.

The one-dimensional TLC method, and the two detection methods, were described by Regev and Cone (1975b).

Two-Dimensional TLC Method

A second TLC method was developed which achieved greater separation of the materials in the crude plant extracts (Fig. 12). Two spots of plant extract were placed on a 20-cm square TLC plate. One of the spots was placed 2 cm from the left-hand margin of the plate, and the other was placed 4 cm from the right-hand margin. The plate was migrated 15 cm in the PE:DE solvent system described above. The plate was dried, and two spots of farnesol standard (usually 15 μg each) were added. One farnesol spot was placed on the origin of the left-hand plant spot, and the other was placed directly above the first, 2 cm from the left-hand margin and 4 cm from the top of the plate. The plate was then turned counter-clockwise, so that the two newly added farnesol spots lay along the bottom edge of the plate. The plate was migrated 14 cm in a benzene-ethyl acetate-acetic acid (BEA) (100:30:3) solvent system, dried, and treated with H_2SO_4 and heat as described above.

The plant sample originally placed near the left-hand margin of the plate was migrated twice, with the migrations running at right angles to each other, resulting in a two-dimensional array of spots. The plant extract originally placed near the right-hand edge

of the plate was migrated only once, allowing a comparison of the two TLC methods. The three farnesol standards on the plate pinpointed the location of the farnesol region in the two-dimensional array.

Gas-Liquid Chromatography Techniques

A gas-liquid chromatography (GLC) method of identifying farnesol was developed, using N-Trifluoroacetyl Imidazole (TFAI) to assist detection.

All GLC analyses were conducted on a Moduline® Varian Aerograph (series 2700) equipped with a 6' x 1/8" I.D. glass column of a 1.4:1 mixture (wt/wt) of 7% QF=1 and 7% DC11, coated on 100/120 mesh high performance (HP) chromosorb W. The following temperatures were used: injector, 200°C; column, 145°C; detector, 235°C. Nitrogen was used as the carrier gas.

Standards were prepared by placing a known amount of farnesol in 2 ml dry benzene. After addition of TFAI (diluted to 10 µl/ml in dry benzene), the samples were heated in a 67°C water bath, and cooled to room temperature. Methanol (0.4 ml) was added to each sample, and thoroughly mixed with a pipette. The sample was then washed three times, using 1 ml of distilled water for each wash. After each wash, the sample was centrifuged and the water fraction was removed with a syringe. Sodium sulfate was added after the

final water wash to dry the sample. The GLC injection size was consistently 2 μl.

Petroleum ether extracts of hop foliage were analyzed using the GLC method described above. Most extracts were partially purified by spotting the crude ether extracts on a TLC plate and migrating them using the one-dimensional TLC method. A farnesol standard was spotted on the margin of each plate, and the R_f of the standard was determined by exposing the margin of the plate to iodine vapor. The TLC gel in the farnesol region of each plant migration was scraped from the plate and washed in benzene. The benzene was separated from the gel by centrifugation, concentrated to 2 ml under a jet of nitrogen, and prepared for GLC injection.

Some plant samples were prepared for GLC analysis without the one-dimensional TLC method; these samples were evaporated to dryness under a jet of nitrogen, redissolved in dry benzene, and prepared for GLC injection using the methods described above.

Column Chromatography Methods

Column chromatography was used in an attempt to concentrate the farnesol in the crude petroleum ether extracts and to eliminate extraneous materials from the extracts.

A slurry of silica gel G (20 g) in petroleum ether was introduced into a 1.8 cm O.D. chromatography column. After washing

the column with 150 ml petroleum ether, a crude petroleum ether extract was placed on the column, and the column was washed with 150 ml petroleum ether. The succeeding washes consisted of 150 ml 10% and 25% diethyl ether in petroleum ether, followed by 150 ml of diethyl ether. Each wash was collected separately and allowed to evaporate to dryness. The residues left after evaporation were redissolved in benzene, evaporated to 2 ml using a jet of nitrogen, and prepared for GLC analysis.

In a separate experiment, the same procedures were followed, but the column was prepared with hexane and washed with diethyl ether in hexane, in the following amounts and proportions: 125 ml ether, 150 ml 10% ether, and 150 ml 100% ether. The washes were collected separately, concentrated to dryness under vacuum, and prepared for GLC analysis.

The column chromatography methods used were similar to those described by Regev and Cone (1975a, 1976a).

Mite Bioassays

The leaves used in these tests were obtained from lima bean plants grown in a greenhouse. Mites were obtained from two cultures of twospotted spider mites maintained on lima bean plants; one culture was maintained in the greenhouse, under natural summer lighting with a mean temperature of 24^{o}C; the other was kept in a

controlled environment room with a temperature of 26°C and a photoperiod of 16 hours light:8 hours darkness.

Oviposition on Farnesol-Treated Leaves

Whole Leaf Test. The stems of mature, uninfested lima bean leaves were placed in vials containing either distilled water (control) or a 1% dilution of farnesol in distilled water. Each leaf was infested with five adult female mites. After five days, the eggs on each leaf were counted.

Leaf Disc Test. Discs 1.23 cm^2 were cut from lima bean leaves and placed on layers of glass wool in petri dishes, with 20 discs/dish. The dishes were filled with distilled water (control), 200 ppm farnesol in water, or 10 ppm farnesol in water. After one day, the discs were removed from the treatment dishes, placed in clean dishes filled with distilled water, and infested. One unmated female mite (four days old) was placed on each disc. Eggs were counted on the second and third days of oviposition. The mites were then moved to fresh, untreated leaf discs, and eggs were counted after two days and after five days of oviposition.

Farnesol in the leaf discs was examined using the one-dimensional TLC method and GLC. Groups of seven uninfested leaf discs treated with 200 ppm farnesol were thoroughly rinsed with water, blotted dry, and shaken with petroleum ether and

anhydrous sodium sulfate. The petroleum ether was concentrated using a jet of nitrogen. The methods used were the same as those described above, except that the sample was not cleaned on a TLC plate before preparation for GLC analysis, and the material was left in petroleum ether, rather than benzene, during the GLC treatment and injection.

Effect of Terpenes on Oviposition

Adult female mites and quiescent female deutonymphs were treated with dilutions of farnesol, nerolidol, geraniol and nerol. All of the dilutions were made in 50% ethanol, and in each test a control group was treated with 50% ethanol. Ca. 0.2 μl of the dilutions were applied topically to each mite, using a small camel's-hair brush (Regev and Cone 1976b). After treatment, the females were placed on 1.23 cm^2 lima bean leaf discs. Unless otherwise stated, one female was placed on each disc. The discs were floated on distilled water in petri dishes containing glass wool. Eggs were counted on each leaf disc.

Two-hundred ppm Standards. Two-hundred ppm dilutions of farnesol, nerolidol, geraniol, and nerol were used to treat adult females chosen at random from the greenhouse mite culture. In a separate experiment, quiescent female deutonymphs from the greenhouse culture were treated with the same materials.

Comparison of Farnesol Dilutions. Adult female mites from the laboratory mite culture were treated with 200 ppm, 100 ppm, or 10 ppm farnesol dilutions. Three mites were placed on each disc, to minimize the effect of individual differences in oviposition rates on the treatment counts.

No Treatment Control Group. Quiescent female deutonymphs from the laboratory mite culture were treated with 200 ppm farnesol or 50% ethanol. A third group, untreated, was used as a control.

In a separate experiment, an untreated control group was compared with quiescent deutonymphs treated with 50% ethanol and 200 ppm farnesol in 50% ethanol. Two dosages were used for the ethanol and farnesol applications. For each material, one group of mites was treated with ca. 0.2 µl, as described in the experiments above, and the other was merely dampened along the dorsum with the treatment material, using a camel's-hair brush.

Effect of Mite Extract on Oviposition

Mites from the greenhouse culture were brushed from lima bean leaves into diethyl ether. A sample of the mites was counted to obtain an estimate of the sex, life stage, and number of mites present. The mites were ground in 20 ml diethyl ether using a microtissue grinder, and the mixture was centrifuged to remove debris. The ether was concentrated under a nitrogen jet to a volume of 5 ml.

An aliquot of the concentrate (0.4 ml), representing about 700 mites (immatures and adults of both sexes) and about 250 eggs, was spotted on a TLC plate. Duplicate plates were prepared, each with standards of farnesol, nerolidol, geraniol, and nerol. One plate was migrated in the PE:DE system, and the other was migrated in the BEA system.

The preparation of the mite extract and the TLC analyses are similar to methods reported by Regev and Cone (1975a).

An aliquot of the extract (3 ml), containing ca. 5000 mites and 2000 eggs, was concentrated to dryness under a nitrogen jet and redissolved in 1 ml 50% ethanol.

Adult female mites from the laboratory culture were treated with the ethanol extract or with 50% ethanol. Two tests were conducted. In the first, 0.2 µl were applied topically to the mites; in the second, the dosage was doubled to 0.4 µl. The concentration of the extract was ca. 5 mites/µl.

Source of Materials Used

Chemicals used in these tests were obtained from the following companies:

Farnesol (mixed isomers): United States Biochemic Corp., Cleveland, OH

Nerolidol, Geraniol, and Nerol (Cis-Geraniol): Pfalz and Bauer, Stamford, CT

N-Trifluoroacetyl Imidazole (TFAI): Pierce, Rockford, IL

Results

Thin-Layer Chromatography

One-Dimensional TLC

The R_f for farnesol using this method was 0.26, and the elution time was ca. 18 min. When a large amount of iodine spray was used to detect standard farnesol solutions, a small spot appeared at an R_f of 0.40, and a very faint spot with an R_f of 0.80 was also evident. The two upper spots were not detectable with H_2SO_4. The main spot (R_f = 0.26) consisted of two lobes which were of about the same size and were confluent, forming a footprint-shaped spot.

Regev and Cone (1975a) also described the two-lobed separation of farnesol on their TLC plates, and suggested that the upper lobe might contain cis-trans farnesol, and that the lower lobe might consist of trans-trans farnesol.

Nerolidol had an R_f of 0.40; the extra spot from farnesol standards with this R_f is probably nerolidol, the structural isomer of farnesol.

Extracts of dried foliage migrated in the single solvent system and treated with H_2SO_4 produced seven distinct spots, with one or two large, dense areas appearing at or very near the R_f of

farnesol. Regions between the spots along the path of migration were also stained, forming a background of varying density, which sometimes obscured the definition of individual spots.

All of the plant extracts analyzed using the single solvent system methods with H_2SO_4 detection appeared to contain farnesol. However, when farnesol was added to plant extracts, the appearance of the migration did not change.

Regev and Cone (1975b) spotted petroleum ether extracts of dried hop leaves on TLC plates, migrated them in a petroleum ether-diethyl ether solvent system, and treated the plates with H_2SO_4 and heat. Farnesol content was determined using a photodensitometer. According to the methods and results they reported (1975b), the photodensitometer could distinguish farnesol quantities ranging from 1.56-3.60 μg in the migrated plant extracts.[3]

In this study, 5 μg was the minimum amount of farnesol which could be confidently detected with the naked eye with the H_2SO_4 detection method. This amount of farnesol could be detected only when the farnesol was migrated alone, without plant extract at the same location. When farnesol was mixed with plant extract, no

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[3]Regev and Cone (1975b) spotted 30 μl of plant extracts containing 0.5 g dry plant foliage/ml petroleum ether on the TLC plates. They reported finding mean farnesol concentrations ranging from 104-240 μg farnesol/g of dry leaf.

difference could be detected between plant extracts containing 5 μg of standard farnesol and extracts without added farnesol.[4] It became apparent that plant materials other than farnesol obscured the farnesol region of the one-dimensional TLC plates when the H_2SO_4 detection method was used.

Using the iodine detection method, plant extracts fortified with farnesol could be distinguished from unfortified extracts. Unfortified plant extracts did not produce stains at the farnesol R_f, but spots did appear in other regions of the plate.

The maximum amount of plant material which could be placed in one spot on the TLC plates was ca. 100 mg (dry plant equivalent). Greater amounts gave retarded and uneven migrations, and the precision of the R_f values was unreliable.

Two-Dimensional TLC

The accuracy of this technique was checked by comparing the migration of plant extracts with and without added farnesol. The standard farnesol in the fortified extracts migrated to a position clearly separated from all of the plant materials (Fig. 12). This

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[4]This observation was based on the migration of 50 μl aliquots of a plant sample containing 1 g dry foliage/ml petroleum ether. The aliquots with added farnesol contained 100 μg of added farnesol/g of dry foliage.

Figure 12. Two-dimensional TLC analysis of a petroleum ether extract of Cascade foliage, with (b) and without (a) farnesol (5 μg) added to the sample.

The spots at the right-hand edge and at the top of each plate are farnesol standards. The long band on the right side of each plate shows the appearance of the migrated plant material (50 mg) after migration using the one-dimensional TLC method; a dense area occurs in the plant band near the farnesol standard to its right.

The scattered spots to the left of the long band show the appearance of the migrated plant material (50 mg) after migration using the two-dimensional TLC method. Farnesol migrates to the circled area. The dense spot which appeared near the farnesol standard in the one-dimensional analysis is found to the right of, and slightly above, the farnesol spot after two-dimensional analysis (b).

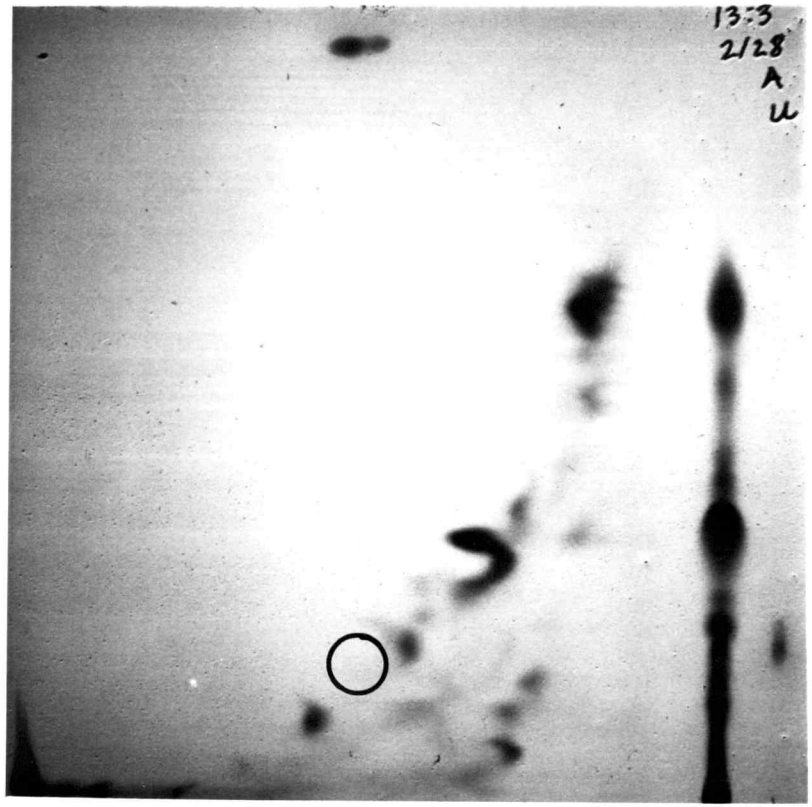

Fig.12A

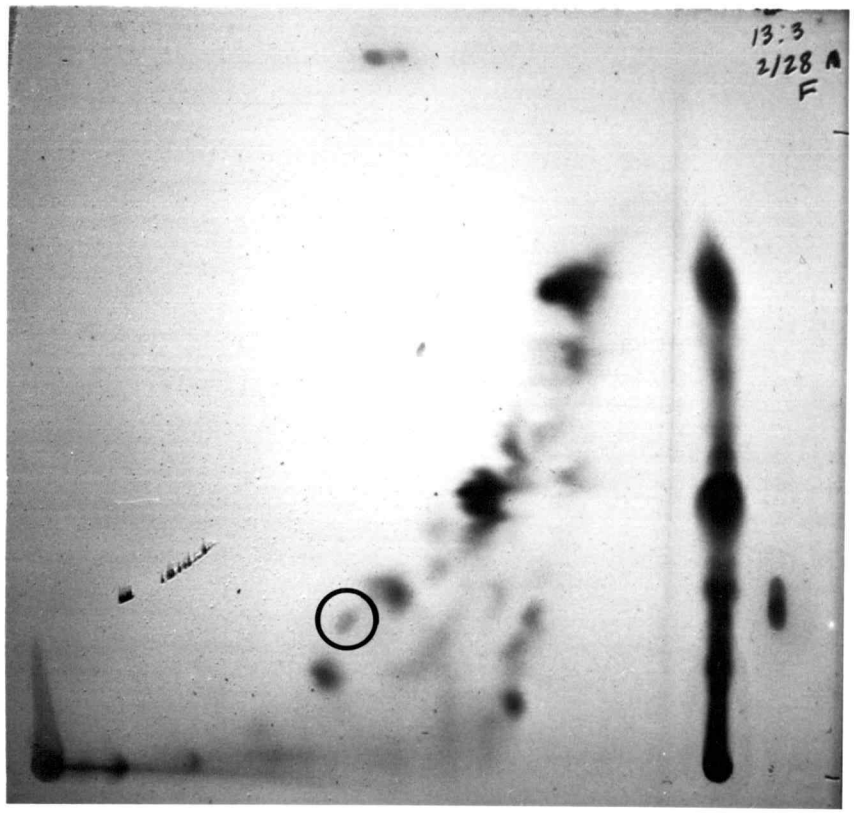

Fig. 12B

proved to be an excellent method for detecting farnesol concentrations in excess of 50 μg/g of dry leaf.

Use of the two-dimensional TLC method provided evidence of the shortcomings of the one-dimensional TLC method for detecting farnesol in plant extracts. In the two-dimensional method, standard farnesol was migrated alone along the edges of the plate to locate the position of the farnesol region. However, when standard farnesol was added to the plant extracts, its position after migration did not align with the position of the farnesol standards on the edges of the plate (Fig. 12). Whenever farnesol was added to plant extracts, its R_f was lower than that of farnesol migrated alone, especially in the first migration of the two-dimensional technique. The R_f of farnesol migrated alone in the first migration was 0.26; the R_f of farnesol added to plant extract was 0.20. Since the solvent used in the first migration is the same as that used in the one-dimensional technique, it was concluded that in either TLC technique, the position of farnesol standards migrated alone does not locate the farnesol region in migrations of plant extract.

The plant material which appeared to be farnesol using the one-dimensional TLC method could be clearly separated from farnesol added to the plant extracts in the two-dimensional TLC method (Fig. 12).

All two-dimensional migrations of plant foliage were conducted using either 25 or 50 μg of plant material, resulting in minimum detection levels of 200 or 100 μg farnesol/g of dry leaf, respectively. Farnesol was not found in any of these samples. When 2.5 mg of Fuggle oil was spotted on the plate, farnesol was detected; the oil contained ca. 5 mg farnesol/g of oil.

Because the standards spotted along the margins of the plate did not accurately locate the farnesol region, it was not feasible to remove the farnesol area for GLC analysis. It was necessary to rely on visual detection methods, with a minimum detection capability of 5 μg of farnesol. Spotting a maximum of 50 mg of plant material on the plate, the minimum detection level was 100 μg/g of dry foliage.

Using the H_2SO_4 detection method, a few foliage samples produced very faint, ill-defined stains in the farnesol or nerolidol regions of the plate. If these stains were farnesol or nerolidol, their concentrations were well below 100 μg/g.

The results of the two-dimensional TLC analyses are presented in Table 12.

GLC Methods

Preliminary Tests

In preliminary experiments, samples containing known amounts of farnesol were treated with varying amounts of TFAI, using

Table 12. Hop samples analyzed for farnesol using the two-dimensional TLC method.

Variety	Farnesol Content (μg/g dry weight)[a]	Previously Reported Farnesol Content (μg/g dry foliage)[b]	Minimum Detection Level for Test (μg farnesol/ g dry weight)
Fuggle Cross (20°C)[c]	--[d]	--[e]	200
Fuggle (120°C)	--	--	100
Fuggle H Cones (20°C)	--	--	200
Comet (62°C, 120°C)	--	240	100
Cascade (62°C, 20°C, wet)	--	192	100, 200
E-2 (120°C)	--	--	200
Talisman (120°C)	--	--	200
L-1 (62°C)	--	124	100
L-16 (120°C)	--	--	100
Fuggle oil	ca. 5000	--	2500

[a] All samples were determined on a dry weight basis except the Fuggle oil sample, which was determined on the basis of μg farnesol/g of oil.

[b] Mean concentration of farnesol in petroleum ether extracts of hop foliage dried at 130°C as reported by Regev and Cone (1975b).

[c] Numbers in parentheses are the temperatures at which the samples were dried. "Wet" designates samples prepared by the wet extraction method.

[d] No farnesol detected; if farnesol was present in the sample, its concentration was less than that indicated in the last column.

[e] No data available.

weight ratios (TFAI:farnesol) of 1:1, 5:1, 7.5:1, 10:1, and 15:1. Two farnesol levels, resulting in 100 and 200 ng GLC injections, were used. With both farnesol levels, it was concluded that maximum farnesol detection was achieved with ratios of 10:1 or greater.

The effect of heating the samples after addition of TFAI was also tested. Samples containing a known amount of farnesol (with TFAI and farnesol in a 10:1 ratio) were heated in a 67°C water bath. No difference in farnesol detection was found using heat treatment lengths varying from 10 min to 2 hr.

Preliminary analyses of standards showed that three farnesol peaks, presumably representing the farnesol isomers cis-trans, trans-trans, and cis-cis (Bates et al. 1962), were detected. The peaks appeared at retention times of 3.1, 3.7, and 4.4 min.

An artifact peak occasionally appeared at a retention time of 3.9 min. Its appearance was independent of the presence of farnesol. When present in samples containing farnesol, it interfered with detection and quantification of the second farnesol peak. The source of the artifact peak was not identified, but it could be eliminated by treatment of the sample with methanol.

A standard curve was prepared for farnesol, using injections ranging from 25 to 200 ng.

When standard farnesol was migrated using the one-dimensional TLC method, scraped from the plate, and prepared for

GLC analysis, an average of 70% of the farnesol was recovered and detected, with no change in the isomeric proportions. Using the same procedures, farnesol was also recovered from fortified plant samples.

Analysis of Plant Extracts

Farnesol was not conclusively identified in any of the foliage samples tested using GLC analysis (Table 13). In some samples, a small peak appeared at a retention time of between 3.7 and 3.9 min (Fig. 13). This material could have been either the second farnesol isomer, or the contaminant noted in the preliminary tests. If this material was farnesol, its concentration in the plant samples was much lower than the level which could be quantified with these methods.

A sample of Fuggle oil cleaned on a TLC plate and analyzed with the GLC contained the third isomer of farnesol (retention time 4.4 min) in a concentration of ca. 250 μg farnesol/g of oil.

Column Chromatography

Petroleum Ether-Diethyl Ether Washes

When standard farnesol was run through the column, farnesol was recovered only in the 25% and 100% diethyl ether washes. Most of the farnesol recovered (77%) was found in the 25% diethyl ether

Figure 13. GLC analysis of (A) 25 ng farnesol standard, (B) 500 mg Cascade foliage, (C) 500 mg Cascade foliage with 25 ng of added farnesol.

Retention times for each of the three farnesol isomers are indicated by the numbers 1-3. Each sample was cleaned on a TLC plate prior to GLC analysis.

FIGURE 13 A, B, C

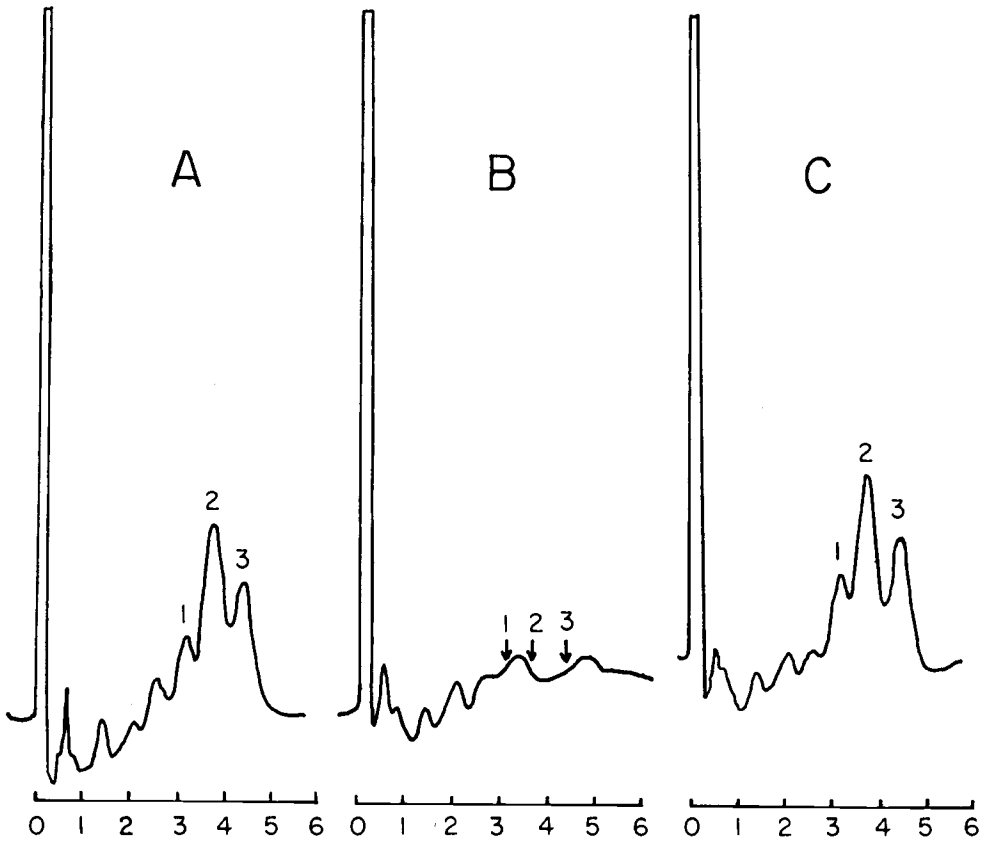

Table 13. Samples analyzed for farnesol using gas-liquid chromatography after cleaning the sample on a TLC plate.

Variety	Farnesol Content (μg/g dry foliage)	Previously Reported Farnesol Content (μg/g dry foliage)[a]	Minimum Detection Level for Test (μg farnesol/ g dry foliage)
Cascade (62°C, 49°C, 180°C)[b]	--[c]	192	8, 9, 50
Fuggle Cross (wet)	--	--[d]	100
Comet Cross (wet)	--	--	17
E-2 (120°C)	--	--	6
Talisman (120°C)	--	--	6
Comet (62°C)	--	192	6
L-1 (62°C)	--	240	6

[a]Mean concentration of farnesol in petroleum ether extracts of hop foliage dried at 130°C as reported by Regev and Cone (1975b).

[b]Numbers in parentheses are the drying temperatures used to prepare the sample. "Wet" indicates samples prepared by the wet extraction method.

[c]No farnesol detected; if farnesol was present in the sample, its concentration was less than that indicated in the last column.

[d]No data available.

wash. The materials recovered produced an abnormal isomeric distribution profile in GLC analysis, and an unidentified contaminant appeared in large quantities at a retention time of 6.8 min.

A sample of Cascade equivalent to 3.6 g of dry leaves was processed in the column and analyzed in the GLC. Using a detection level of 7 μg farnesol/g of dry foliage, no farnesol was identified in the sample. Very small peaks, too low for quantification, appeared in the farnesol region of the GLC charts for all washes except the 100% diethyl ether wash. According to the results from the farnesol standard, the first two washes (0 and 10% diethyl ether in petroleum ether) should not contain farnesol. These peaks apparently represent procedural contaminants, non-target compounds, or very low levels of farnesol. None of the peaks in the farnesol region were large enough to be quantified.

Hexane-Diethyl Ether Washes

This procedure was used for a sample of Cascade foliage (equivalent to 10 g dry foliage) and for the same sample fortified with 25 μg of standard farnesol. A third column, with 25 μg of standard farnesol, was run as a control.

The hexane fractions of the two plant samples could not be analyzed using GLC methods, because the samples emulsified when washed with water. The hexane wash from the column gave a large

peak at a retention time of 3.7 min (the second farnesol isomer), indicating that if the second isomer of farnesol did exist in the plant samples, it may have been lost in the emulsified samples. All of the other washes, from all three treatments, contained very small peaks (below the level of quantification) at a retention time of 3.9 min. The large peaks had the same retention properties as the contaminant previously encountered in some GLC samples.

Mite Bioassays

Oviposition on Farnesol-Treated Leaves

In all of the concentrations used, the farnesol did not dissolve in the distilled water. The water fraction became cloudy, but most of the farnesol floated on top of the water fraction. The leaf discs turned white and became flaccid where they contacted the farnesol. In the whole leaf test, some of the farnesol was translocated into the leaves, turning the veins and portions of the interveinal areas white.

Whole Leaf Test. Mites on leaves treated with 1% farnesol in distilled water laid an average of 100.5 eggs/leaf; those on the control leaves laid an average of 107.0 eggs/leaf. The means are not significantly different, using analysis of variance ($P = 0.05$).

Leaf Disc Test. No significant differences ($P = 0.05$) were found in oviposition of mites confined on discs treated with farnesol

in distilled water (10 ppm and 200 ppm) or controls treated with distilled water. After five days of oviposition, the mean number of eggs deposited were: 10 ppm farnesol, 23.71 eggs; 200 ppm farnesol, 24.36 eggs; control, 24.50 eggs.

The leaf discs treated with 200 ppm farnesol contained ca. 6-10 mg farnesol/g of dry leaf.

Effect of Terpenes on Oviposition

None of the terpene dilutions dissolved in 50% ethanol. The ethanol fractions became cloudy, but most of the farnesol separated from solution and floated on top of the mixture.

Each set of egg counts was analyzed using analysis of variance. When significant differences were found, the treatment means were compared with the control mean using the Least Significant Difference (LSD) test. All significance tests were conducted with $P = 0.05$.

Two-hundred ppm Terpenes. In the test using adult females, six egg counts were conducted. Significant differences in oviposition were found only on the second day of oviposition. On that day, the mean numbers of eggs per female were: farnesol, 3.25; geraniol, 5.25; ethanol, 5.67; nerol, 6.73; nerolidol, 7.64. None of the terpene treatments differed significantly from the ethanol control.

In the test using quiescent deutonymphs, three egg counts were conducted. Farnesol-treated mites produced significantly fewer eggs ($P = 0.05$) than the control group (Table 14).

Comparison of Farnesol Dilutions. The oviposition of mites treated with 200 ppm, 100 ppm, and 10 ppm farnesol in 50% ethanol did not differ significantly ($P = 0.05$) from the 50% ethanol control group.

No-Treatment Control Group. In the first test, untreated mites laid significantly more eggs ($P = 0.05$) than the mites treated with 50% ethanol. Mites treated with 200 ppm farnesol in 50% ethanol did not differ significantly from the no treatment control group. The mean number of eggs/♀ for each treatment was: 50% ethanol, 7.13 eggs; 200 ppm farnesol, 10.12 eggs; no treatment control group, 12.47 eggs.

In the second test, untreated mites again laid significantly more eggs ($P = 0.05$) than the mites treated with 50% ethanol, and the number of eggs laid appeared to vary with the amount of ethanol applied (for both the 50% ethanol treatments and the 200 ppm farnesol in 50% ethanol treatments, two dosages were applied; one group was merely dampened ("spotted")with the material, while the other received a 0.2 µl dosage). For each treatment group, the mean number of eggs/♀ was: 0.2 µl 50% ethanol, 14.07 eggs; 0.2 µl 200 ppm farnesol, 15.25 eggs; 50% ethanol spot, 16.88 eggs; 200 ppm farnesol spot, 16.47 eggs; no treatment, 18.45 eggs.

Table 14. Mean number of eggs laid by females treated with 200 ppm terpenes as quiescent deutonymphs.

Oviposition day	Treatment				
	Farnesol	Nerol	Geraniol	Nerolidol	Ethanol Control
2	6.57 (14)[a]	8.76 (17)	9.18 (11)	9.43 (14)	9.38 (16)
3	11.29 (14)	14.88 (17)	16.56 (9)	14.93 (14)	16.06 (16)
7	30.08 (12)	40.43 (14)	42.70 (10)	37.83 (12)	39.57 (14)

[a]Numbers in parentheses are the number of females represented in the mean.

Effect of Mite Extract on Mite Oviposition

The concentrated diethyl ether extract of mites was green in color. When the material was dried and washed with 50% ethanol, none of the pigments redissolved. The concentrated ether extract represented an estimated 1753 mites and 617 eggs/ml, and the ethanol concentrate represented about 5259 mites and 1851 eggs/ml. All stages of mites were represented, in the following proportions: 9% adult females, 20% adult males, 15% quiescent female deutonymphs, and 56% immature mites other than quiescent female deutonymphs.

Very faint spots appeared on the TLC plate of the diethyl ether extract near the R_f of farnesol and of geraniol. The materials in this area were not positively identified. Regev and Cone (1975a, 1976a) reported finding farnesol, nerolidol, and geraniol in crude ether extracts of pharate females.

The 50% ethanol concentrate was topically applied to female mites in two tests. No significant differences were found in oviposition comparing the treatment group with a control group treated with 50% ethanol. Female deutonymphs treated with the extract produced normal appearing adults; no evidence of juvenile hormone effects (e.g., retention of immature characteristics of failure to ecdyse) was noted in the treated mites.

Discussion

Two of the analytical methods reported here are of value. The two-dimensional TLC method is useful for qualitative detection of farnesol in concentrations greater than 50 μg/g of dry leaf. GLC analysis of material scraped from TLC plates will quantitatively detect farnesol concentrations down to 6 μg/g of dry foliage.

None of the hop foliage samples tested had farnesol concentrations sufficiently large to be detected by these methods. If farnesol is to be detected at lower levels, it is necessary to devise a new method for eliminating extraneous materials from the samples and for concentrating the farnesol in the samples. This was attempted with column chromatography, but contaminants appeared in the farnesol region of the column-washed samples.

The one-dimensional TLC method is not suitable for qualitative or quantitative analysis of farnesol in hop foliage, especially using H_2SO_4 as a detection agent. An unidentified plant material other than farnesol migrates to the farnesol region in a single migration, and obscures identification of the farnesol. In addition, when farnesol is added to plant samples, its R_f differs from that of farnesol migrated alone. Spotting farnesol standards alone on the plate will not identify the farnesol region in the plant samples.

The one-dimensional TLC method was used by Regev and Cone (1975b) to analyze petroleum ether extracts of hop foliage. After

charring the plates with H_2SO_4, they measured the density of the farnesol region in the plant extracts using a photodensitometer. The farnesol concentrations they reported (1975b) ranged from 104-240 μg/g of dry foliage. It is very likely that the photodensitometer measured the density of the plant material other than farnesol which migrated to the farnesol region of the one-dimensional plate. The two-dimensional TLC plate showed conclusively that this material is not farnesol.

Since farnesol was found in hop oil, it is likely that it also appears in the foliage of hop plants. However, if farnesol was present in the foliage samples analyzed in this study, its concentration was always below the minimum detection levels used in the analyses.

Regev and Cone (1975b) stated that farnesol levels in hop foliage decreased as the growing season progressed. Since all of the foliage samples analyzed in this study were collected in September, it is possible that the farnesol contents of the leaves were very low due to the lateness of the collection date. However, the very low detection levels used in some of the analyses (i.e. 6 ppm) ought to have compensated for this.

The bioassays did not produce evidence of farnesol acting as a gonadotropin. When significant differences did occur, oviposition rates were lower in farnesol-treated mites than in ethanol controls.

Mites exposed to massive amounts of farnesol in the leaf treatment tests did not differ from control groups in oviposition rates.

In tests of the mite extract, no evidence was found to support the hypothesis that an ethanol-soluble gonadotropin was available in mite tissues.

It was extremely difficult to administer precise dosages of materials to the mites. A large degree of variation probably occurred within treatment groups when topically applying the terpenes, mite extract, and ethanol. In addition, systematic errors in dosage probably occurred because a different brush was used to apply each treatment solution; this may result in a different dosage of ethanol being applied to each treatment group. Topical applications were abandoned when it was found that ethanol alone will significantly reduce mite oviposition rates.

All of the terpene dilutions used were actually saturated solutions. This causes further problems in dosage control, since the brush may contact an oil droplet when dipped into the dilution, and since the concentration of the dilution will depend on its temperature.

All of the terpenes used in this study are extremely aromatic. Carlisle et al. (1965), in a study of desert locusts, suggested that the odor of certain terpenes is sufficient to stimulate vitellogenesis and oocyte growth in female locusts. If twospotted spider mites

were equally sensitive to the odors of the compounds used, the control groups would probably be stimulated as much as the treatment groups, since they were all kept in the same controlled environment room.

The aromatic nature of terpenes also interferes with quantitative analysis. Standards of nerol and gernaiol were difficult to detect on TLC plates because the materials rapidly evaporated off of the plates. When farnesol was added to powdered hop foliage and heated at 180^{o}C more than 90% of the farnesol was lost; because of the aromatic nature of these materials, heating of samples is not recommended.

The results of this study do not support the hypothesis that farnesol is related to the resistance of hop varieties to the two-spotted spider mite.

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APPENDIX I

Simple Linear Regressions of Mite Life History Data and Hop Leaf Characteristics

The regression equation is given for each regression, followed by the F_s value and degree of freedom from analysis of variance, and the R^2 value. Methods are presented in Chapter V, under the heading "Effect of Varietal Characteristics on Mite Life History."

Y variables: Eggs, survival, development time (of all progeny), female development time (of female progeny only), and female ratio (female progeny/total progeny).

X variables: Leaf area, leaf weight ratio (dry weight/wet weight), leaf hairs, vein hairs, and glands.

Regressions of Pooled Data

Data from varieties Cascade, Comet, Fuggle, L-16, and Talisman were combined for these regressions.

Eggs = $7.0137 + 4.2165 \times 10^{-2}$ Leaf area

$P \leq 0.025$ $F_s = 6.95$ df = 1, 61

$R^2 = 0.10227143$

Eggs = 9.0936 - 1.6126 Leaf weight ratio

NS $F_s = 0.02$ df = 1, 61

$R^2 = 0.00035002$

Eggs = $8.6430 - 1.9345 \times 10^{-2}$ Leaf hairs

NS $F_s = 0.64$ df = 1, 61

$R^2 = 0.01039457$

Eggs = $8.9886 - 3.3495 \times 10^{-2}$ Vein hairs

NS $F_s = 1.16$ df = 1, 61

$R^2 = 0.01873619$

Eggs = $8.9586 - 3.3875 \times 10^{-3}$ Glands

NS $F_s = 0.33$ df = 1, 61

$R^2 = 0.00539547$

Survival = $6.3567 \times 10^{-1} + 2.0828 \times 10^{-3}$ Leaf area

NS $F_s = 1.01$ df = 1, 50

$R^2 = 0.01978409$

Survival = $6.6641 \times 10^{-1} + 1.2379 \times 10^{-1}$ Leaf weight ratio

NS $F_s = 0.01$ df = 1, 50

$R^2 = 0.00015763$

Survival = $7.0997 \times 10^{-1} + 1.4435 \times 10^{-4}$ Leaf hairs

NS $F_s = 0.002$ df = 1, 50

$R^2 = 0.00004743$

Survival = $7.5021 \times 10^{-1} - 2.6185 \times 10^{-3}$ Vein hairs

NS $F_s = 0.44$ df = 1, 50

$R^2 = 0.00882736$

Survival = $6.7426 \times 10^{-1} + 2.7947 \times 10^{-4}$ Glands

NS $F_s = 0.13$ df = 1, 50

$R^2 = 0.00253627$

Female ratio = $8.3954 \times 10^{-1} - 1.3675 \times 10^{-3}$ Leaf area

NS $F_s = 0.48$ df = 1, 60

$R^2 = 0.00791759$

Female ratio = $8.9268 \times 10^{-1} - 2.8022 \times 10^{-1}$ Leaf weight ratio

NS $F_s = 0.05$ df = 1, 60

$R^2 = 0.00078047$

Female ratio = $7.4071 \times 10^{-1} + 7.1400 \times 10^{-3}$ Leaf hairs

$P \leq 0.025$ $F_s = 7.00$ df = 1, 60

$R^2 = 0.10446530$

Female ratio - $6.8571 \times 10^{-1} + 7.3208 \times 10^{-3}$ Vein hairs

$P \leq 0.05$ $F_s = 4.25$ df = 1, 60

$R^2 = 0.06608607$

Female ratio = $7.2109 \times 10^{-1} + 5.2651 \times 10^{-4}$ Glands

NS $F_s = 0.58$ df = 1, 60

$R^2 = 0.00957365$

Development time = $1.2596 \times 10^{1} + 1.0986 \times 10^{-3}$ Leaf area

NS $F_s = 0.08$ df = 1, 60

$R^2 = 0.00137925$

Development time = $1.0744 \times 10^{1} + 5.2082$ Leaf weight ratio

$P \leq 0.05$ $F_s = 4.71$ df = 1, 60

$R^2 = 0.07276676$

Development time = $1.2538 \times 10^{1} + 1.3888 \times 10^{-2}$ Leaf hairs

$P \leq 0.01$ $F_s = 7.17$ df = 1, 60

$R^2 = 0.10667962$

Development time = $1.2622 \times 10^{1} + 9.1984 \times 10^{-4}$ Vein hairs

NS $F_s = 0.02$ df = 1, 60

$R^2 = 0.00028160$

Development time = $1.2413 \times 10^{1} + 1.6758 \times 10^{-3}$ Glands

NS $F_s = 1.61$ df = 1, 60

$R^2 = 0.02617622$

Female development time = $1.2837 \times 10^{1} - 4.9891 \times 10^{-4}$ Leaf area

NS $F_s = 0.02$ df = 1, 59

$R^2 = 0.00028665$

Female Development time = $1.1002 \times 10^{1} + 5.0023$ Leaf weight ratio

$P \leq 0.05$ $F_s = 4.28$ df = 1, 59

$R^2 = 0.06759764$

Female Development Time = $1.2767 \times 10^{1} + 7.3293 \times 10^{-3}$ Leaf hairs

NS $F_s = 1.81$ df = 1, 59

$R^2 = 0.02973473$

Female development time = $1.2827 \times 10^{1} - 5.3409 \times 10^{-4}$ Vein hairs

NS $F_s = 0.01$ df = 1, 59

$R^2 = 0.00009276$

Female Development Time = $1.2669 \times 10^{1} + 1.1206 \times 10^{-3}$ Glands

NS $F_s = 0.67$ df = 1, 59

$R^2 = 0.01124382$

Regressions of Varietal Data

When significant regressions were obtained from the pooled data (analysis of variance, P = 0.05), the data were separated by varieties and regressed again.

Cascade Variety

Eggs = $7.2886 + 5.3354 \times 10^{-2}$ Leaf area

NS $F_s = 0.67$ df = 1, 11

$R^2 = 0.05728530$

Female Ratio = $9.4139 \times 10^{-1} - 2.8868 \times 10^{-2}$ Vein hairs

NS $F_s = 1.35$ df = 1, 11
$R^2 = 0.10963549$

Female ratio = $7.4817 \times 10^{-1} + 1.5418 \times 10^{-2}$ Leaf hairs

NS $F_s = 0.18$ df = 1, 11
$R^2 = 0.01578982$

Development time = $1.2002 \times 10^{1} + 2.0547$ Leaf weight ratio

NS $F_s = 0.11$ df = 1, 11
$R^2 = 0.01024097$

Development time = $1.2816 \times 10^{1} - 4.9318 \times 10^{-2}$ Leaf hairs

NS $F_s = 0.31$ df = 1, 11
$R^2 = 0.02714012$

Female development time = $1.3221 \times 10^{1} - 7.9223 \times 10^{-1}$ Leaf weight ratio

NS $F_s = 0.02$ df = 1, 11
$R^2 = 0.00148235$

Comet Variety

Eggs = $8.1350 + 2.9323 \times 10^{-2}$ Leaf area

NS $F_s = 3.56$ df = 1, 13
$R^2 = 0.21503154$

Female ratio = $7.7289 \times 10^{-1} + 1.3908 \times 10^{-3}$ Vein hairs

NS $F_s = 0.02$ df = 1, 12
$R^2 = 0.00185921$

Female ratio = $7.9465 \times 10^{-1} + 3.6311 \times 10^{-4}$ Leaf hairs

NS $F_s = 0.001$ df = 1, 12
$R^2 = 0.00008617$

Development time = $1.2736 \times 10^{1} - 5.7211 \times 10^{-1}$ Leaf weight ratio

NS $F_s = 0.02$ df = 1, 12
$R^2 = 0.00127276$

Development time = $1.2447 \times 10^{1} + 2.5738 \times 10^{-2}$ Leaf hairs

NS $F_s = 1.22$ df = 1, 12
$R^2 = 0.09240987$

Female development time = $1.1540 \times 10^{1} + 3.0614$ Leaf weight ratio

NS $F_s = 0.37$ df = 1, 12
$R^2 = 0.02974435$

Fuggle Variety

Eggs = $7.0140 + 2.5314 \times 10^{-2}$ Leaf area

NS $F_s = 0.10$ df = 1, 9
$R^2 = 0.01048034$

Female ratio = $6.4354 \times 10^{-1} + 8.5803 \times 10^{-3}$ Vein hairs

NS $F_s = 0.26$ df = 1, 9
$R^2 = 0.02776356$

Female ratio = $5.7091 \times 10^{-1} + 9.5589 \times 10^{-2}$ Leaf hairs

NS $F_s = 2.68$ df = 1, 9
$R^2 = 0.22971750$

Development time = $8.1008 + 1.2157 \times 10^{1}$ Leaf weight ratio

NS $F_s = 1.76$ df = 1, 9

$R^2 = 0.16347418$

Development time = $1.2720 \times 10^{1} + 6.3235 \times 10^{-3}$ Leaf hairs

NS $F_s = 0.005$ df = 1, 9

$R^2 = 0.00051407$

Female development time = $1.0127 \times 10^{1} + 7.4906$ Leaf weight ratio

NS $F_s = 1.08$ df = 1, 8

$R^2 = 0.11920303$

L-16 Variety

Eggs = $7.3288 - 1.6141 \times 10^{-3}$ Leaf area

NS $F_s = 0.001$ df = 1, 12

$R^2 = 0.00007477$

Female ratio = $6.7916 \times 10^{-1} + 8.7344 \times 10^{-3}$ Vein hairs

NS $F_s = 0.52$ df = 1, 12

$R^2 = 0.04159194$

Female ratio = $7.4927 \times 10^{-1} + 7.8119 \times 10^{-3}$ Leaf hairs

NS $F_s = 1.31$ df = 1, 12

$R^2 = 0.09814235$

Development time = $1.3377 \times 10^{1} - 2.0685$ Leaf weight ratio

NS $F_s = 0.08$ df = 1, 12

$R^2 = 0.00641746$

Development time = $1.2361 \times 10^{1} + 2.0561 \times 10^{-2}$ Leaf hairs

$P \leq 0.05$ $F_s = 5.87$ df = 1, 12
$R^2 = 0.32846695$

Female development time = $1.4718 \times 10^{1} - 5.2165$ Leaf weight ratio

NS $F_s = 0.45$ df = 1, 12
$R^2 = 0.03613369$

Talisman Variety

Eggs = $1.4085 \times 10^{1} - 1.2932 \times 10^{-1}$ Leaf area

$P \leq 0.05$ $F_s = 6.97$ df = 1, 8
$R^2 = 0.46573468$

Female ratio = $1.3206 \times 10^{-2} + 3.6304 \times 10^{-2}$ Vein hairs

$P \leq 0.025$ $F_s = 7.70$ df = 1, 8
$R^2 = 0.49058557$

Female ratio = $7.0658 \times 10^{-1} + 6.7955 \times 10^{-3}$ Leaf hairs

NS $F_s = 3.02$ df = 1, 8
$R^2 = 0.27372012$

Development time = $7.1867 + 1.5701 \times 10^{1}$ Leaf weight ratio

$P \leq 0.05$ $F_s = 6.79$ df = 1, 8
$R^2 = 0.45918394$

Development time = $1.1810 \times 10^{1} + 4.1225 \times 10^{-2}$ Leaf hairs

$P \leq 0.025$ $F_s = 10.86$ df = 1, 8
$R^2 = 0.57573591$

Female Development time = $7.2294 + 1.5926 \times 10^{1}$ Leaf weight ratio

$P \leq 0.025$ $F_s = 8.79$ df = 1, 8

$R^2 = 0.52363448$

APPENDIX II

Multiple Linear Regressions of Mite Life History Data and Hop Leaf Characteristics

X variables were added to the regression in stepwise order, from best to worst regression fits. The regression equation is given for each regression, followed by the F_s value and degrees of freedom from analysis of variance, and the R^2 value. Methods are presented in Chapter V, under the heading "Effects of Varietal Characteristics on Mite Migration."

Y variables: Eggs, survival, development time (of all progeny), female development time (of female progeny only), and female ratio (female progeny/total progeny).

X variables: Leaf area, leaf weight ratio (dry weight/wet weight), leaf hairs, vein hairs, and glands.

Eggs = 8.5079

Eggs = $7.0137 + 4.2165 \times 10^{-2}$ Leaf area

$P \leq 0.025$ $F_s = 6.95$ df = 1, 61

$R^2 = 0.10227143$

Eggs = $7.5508 + 4.7043 \times 10^{-2}$ Leaf area $- 4.9474 \times 10^{-2}$ Vein hairs

$P \leq 0.025$ $F_s = 4.96$ df = 2, 60

$R^2 = 0.14177814$

Eggs = $8.1403 + 4.8920 \times 10^{-2}$ Leaf area - 4.8089×10^{-2} Vein hairs - 5.0800×10^{-3} Glands

$P \leq 0.025$ $F_s = 3.57$ df = 3, 59

$R^2 = 0.15364768$

Eggs = $8.1445 + 4.9441 \times 10^{-2}$ Leaf area - 1.5025×10^{-2} Leaf hairs - 3.8700×10^{-2} Vein hairs - 5.4745×10^{-3} Glands

$P \leq 0.05$ $F_s = 2.73$ df = 4, 58

$R^2 = 0.15836025$

Eggs = $9.6124 + 4.9309 \times 10^{-2}$ Leaf area - 4.0318 Leaf weight ratio - 1.5165×10^{-2} Leaf hairs - 4.2110×10^{-2} Vein hairs - 5.0912×10^{-3} Glands

NS $F_s = 2.18$ df = 5, 57

$R^2 = 0.16028699$

Survival = 7.1114×10^{-1}

Survival = $6.3567 \times 10^{-1} + 2.0828 \times 10^{-3}$ Leaf area

NS $F_s = 1.01$ df = 1, 50

$R^2 = 0.01978409$

Survival = $6.7518 \times 10^{-1} + 2.3112 \times 10^{-3}$ Leaf area - 3.2025×10^{-3} Vein hairs

NS $F_s = 0.83$ df = 2, 49

$R^2 = 0.03275035$

Survival = $6.7880 \times 10^{-1} + 2.2686 \times 10^{3}$ Leaf area + 1.2891 $\times 10^{-3}$ Leaf hairs - 4.0411×10^{-3} Vein hairs

NS $F_s = 0.59$ df = 3,48

$R^2 = 0.03559605$

Survival = $6.5181 \times 10^{-1} + 2.1764 \times 10^{-3}$ Leaf area + 1.4141 $\times 10^{-3}$ Leaf hairs - 4.1714×10^{-3} Vein hairs + 2.3695×10^{-4} Glands

NS $F_s = 0.46$ df = 4,47

$R^2 = 0.03735125$

Survival = $7.6510 \times 10^{-1} + 2.1616 \times 10^{-3}$ Leaf area - 3.1002×10^{-1} Leaf weight ratio + 1.4431×10^{-3} Leaf hairs - 4.4980×10^{-3} Vein hairs + 2.6657×10^{-4} Glands

NS $F_s = 0.36$ df = 5,46

$R^2 = 0.03819456$

Female ratio = 7.9092×10^{-1}

Female ratio = $7.4071 \times 10^{-1} + 7.1400 \times 10^{-3}$ Leaf hairs

$P \leq 0.025$ $F_s = 7.00$ df = 1,60

$R^2 = 0.10446530$

Female ratio = $8.0942 \times 10^{-1} - 2.0017 \times 10^{-3}$ Leaf area + 7.4902×10^{-3} Leaf hairs

$P \leq 0.005$ $F_s = 9.62$ df = 2,59

$R^2 = 0.12117865$

Female ratio = $7.1851 \times 10^{-1} - 2.3362 \times 10^{-3}$ Leaf area
$+ 7.7435 \times 10^{-3}$ Leaf hairs $+ 7.6169 \times 10^{-4}$
Glands

$P \leq 0.05$ $F_s = 3.16$ df = 3.58

$R^2 = 0.14067086$

Female ratio = $6.8913 \times 10^{-1} - 2.5751 \times 10^{-3}$ Leaf area
$+ 6.2667 \times 10^{-3}$ Leaf hairs $+ 3.9879 \times 10^{-3}$
Vein hairs $+ 6.9342 \times 10^{-4}$ Glands

$P \leq 0.05$ $F_s = 2.62$ df = 4, 57

$R^2 = 0.15520596$

Female ratio = $5.4614 \times 10^{-1} - 2.5621 \times 10^{-3}$ Leaf area
$+ 3.9274 \times 10^{-1}$ Leaf weight ratio + 6.2804
$\times 10^{-3}$ Leaf hairs $+ 4.3201 \times 10^{-3}$ Vein hairs
$+ 6.5604 \times 10^{-4}$ Glands

NS $F_s = 2.08$ df = 5, 56

$R^2 = 0.15655643$

Development time = 1.2635×10^{1}

Development time = $1.2538 \times 10^{1} + 1.3888 \times 10^{-2}$ Leaf hairs

$P \leq 0.01$ $F_s = 7.17$ df = 1, 60

$R^2 = 0.10667962$

Development time = $1.0159 \times 10^{1} + 6.5030$ Leaf weight ratio
$+ 1.6381 \times 10^{-2}$ Leaf hairs

$P \leq 0.005$ $F_s = 8.16$ df = 2, 59

$R^2 = 0.21668649$

Development time = $1.0109 \times 10^{1} + 6.1145$ Leaf weight ratio $+ 1.6597 \times 10^{-2}$ Leaf hairs $+ 1.4277 \times 10^{-3}$ Glands

$P \leq 0.005$ $F_s = 5.95$ df = 3, 58

$R^2 = 0.23521950$

Development time = $1.0382 \times 10^{1} + 5.5244$ Leaf weight ratio $- 7.0189 \times 10^{-3}$ Vein hairs $+ 1.6152 \times 10^{-3}$ Glands $+ 1.9061 \times 10^{-2}$ Leaf hairs

$P \leq 0.005$ $F_s = 4.67$ df = 4, 57

$R^2 = 0.24667978$

Development time = $1.0383 \times 10^{1} - 4.9816 \times 10^{-5}$ Leaf area $+ 5.5236$ Leaf weight ratio $+ 1.9065 \times 10^{-2}$ Leaf hairs $- 7.0065 \times 10^{-3}$ Vein hairs $+ 1.6176 \times 10^{-3}$ Glands

$P \leq 0.01$ $F_s = 3.67$ df = 5, 56

$R^2 = 0.24668246$

Female Development Time = 1.2819×10^{1}

Female development time = $1.1002 \times 10^{1} + 5.0023$ Leaf weight ratio

$P \leq 0.05$ $F_s = 4.28$ df = 1, 59

$R^2 = 0.06759764$

Female development time = $1.0654 \times 10^{1} + 5.7717$ Leaf weight ratio $+ 9.5892 \times 10^{-3}$ Leaf hairs

$P \leq 0.05$ $F_s = 3.84$ df = 2, 58

$R^2 = 0.11689705$

Female development time = 1.0617×10^{1} + 5.5520 Leaf weight ratio + 9.7912×10^{-3} Leaf hairs + 8.6560×10^{-4} Glands

NS $F_s = 2.68$ df = 3, 57

$R^2 = 0.12343353$

Female development time = 1.0667×10^{1} - 1.2803×10^{-3} Leaf area + 5.5062 Leaf weight ratio + 1.0022×10^{-2} Leaf hairs + 9.4084×10^{-4} Glands

NS $F_s = 2.00$ df = 4, 56

$R^2 = 0.12524214$

Female development time = 1.0760×10^{1} - 1.1444×10^{-3} Leaf area + 5.3033 Leaf weight ratio + 1.0837×10^{-2} Leaf hairs - 2.4544×10^{-3} Vein hairs + 9.8613×10^{-4} Glands

NS $F_s = 1.59$ df = 5, 55

$R^2 = 0.12660666$

APPENDIX III

Simple Linear Regressions of Mite Migration Data and Hop Leaf Characteristics

The regression equation is given for each regression, followed by the F_s value and degrees of freedom from analysis of variance, and the R^2 value. Methods for these regressions are presented in Chapter V, under the heading "Effect of Varietal Characteristics on Mite Migration."

Y variable: Number of mites on each leaf disc after migration.

X variable: Leaf area, leaf weight ratio (dry weight/wet weight), leaf hairs, vein hairs, and glands. The leaf weight ratio and leaf hairs were log-transformed ($\log_e X + 1$).

Mites = $1.0011 \times 10^{1} + 4.0155 \times 10^{-2}$ Leaf area

NS $F_s = 0.06$ df = 1, 13

$R^2 = 0.00483782$

Mites = $7.5794 \times 10^{1} - 2.1042 \times 10^{2}$ Leaf weight ratio

NS $F_s = 2.69$ df = 1, 13

$R^2 = 0.17137440$

Mites = 9.1717 + 1.6249 Leaf hairs

NS $F_s = 0.42$ df = 1, 13

$R^2 = 0.03155853$

Mites = $1.8032 \times 10^{1} - 2.5870 \times 10^{-1}$ Vein hairs

NS $F_s = 2.47$ df = 1, 13

$R^2 = 0.15965051$

Mites = $1.2265 \times 10^{1} - 1.3512 \times 10^{-3}$ Glands

NS $F_s = 0.001$ df = 1, 13

$R^2 = 0.00004782$

APPENDIX IV

Multiple Linear Regressions of Mite Migration Data and Hop Leaf Characteristics

X variables were added to the regression in stepwise order, from best to worst regression fits. The regression equation is given for each regression, followed by the F_s value and degrees of freedom from analysis of variance, and the R^2 value. Methods for these regressions are presented in Chapter V, under the heading "Effects of Varietal Characteristics on Mite Migration."

Y variable: Number of mites on each leaf disc after migration.

X variables: Leaf area, leaf weight ratio (dry weight/wet weight), leaf hairs, vein hairs, and glands. The leaf weight ratio and leaf hairs were log-transformed ($\log_e X + 1$).

$$\text{Mites} = 1.2133 \times 10^{1}$$

$$\text{Mites} = 7.5794 \times 10^{1} - 2.1042 \times 10^{2} \text{ Leaf weight ratio}$$

NS $\quad F_s = 2.69 \quad df = 1, 13$

$R^2 = 0.17137440$

$$\text{Mites} = 7.7951 \times 10^{1} - 1.9917 \times 10^{2} \text{ Leaf weight ratio} - 2.4380 \times 10^{-1} \text{ Vein hairs}$$

NS $\quad F_s = 2.73 \quad df = 2, 12$

$R^2 = 0.31266947$

Mites = $7.6975 \times 10^{1} - 2.1215 \times 10^{2}$ Leaf weight ratio
$+ 4.7042$ Leaf hairs $- 4.0479 \times 10^{-1}$ Vein hairs

$P \leq 0.05$ $F_s = 3.88$ df = 3, 11

$R^2 = 0.51395902$

Mites = $7.3981 \times 10^{1} + 2.3841 \times 10^{-2}$ Leaf area $- 2.0635 \times 10^{2}$
Leaf weight ratio $+ 4.7445$ Leaf hairs $- 4.0903 \times 10^{-1}$
Vein hairs

NS $F_s = 2.66$ df = 4, 10

$R^2 = 0.51550060$

Mites = $7.2644 \times 10^{1} + 3.3361 \times 10^{-2}$ Leaf area $- 2.0158 \times 10^{2}$
Leaf weight ratio $+ 4.7877$ Leaf hairs $- 4.1290 \times 10^{-1}$
Vein hairs $- 6.1370 \times 10^{-3}$ Glands

NS $F_s = 1.92$ df = 5, 9

$R^2 = 0.51620733$