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Management of Bulb Mites (Rhizoglyphus Spp.) through a Study of their Biology and Ecology

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ABSTRACT

The mite Rhizoglyphus robini damaged onion and garlic in Israel during autumn. Manure applications increased injury incidence. Monitoring by traps showed mite activity in the soil from fall to spring, continuing into summer only in irrigated plots. Brazilian garlic was tolerant, but no correlations were obtained between mite numbers and damage sustained by another variety. Analyses of mite-harboring soils revealed no consistent pattern. Mites lived for several weeks and deposited 690 eggs/female on peanut seeds; population increase was also enhanced on manures, roots and macerated soil invertebrates. Rhizoglyphus preferred the soil fungus Fusarium oxysporum to four others and the largest populations developed when mites fed on it. Hypopodes molted best at 24°C and 100% r.h. Length of dorsal setae sc i, important for species recognition, was affected by diet richness. Mites survived and retained their fertility after prolonged immersion in water. An enzymatic method was developed for separating mites from plant tissue. Effective mite control was obtained with solar heating of the soil, one chemical disinfectant and some pesticides. It is postulated that Rhizoglyphus is a soil-borne mite whose outbreaks were invoked by agricultural practices. Guidelines are outlined for pest management in the field.

1. Introduction

Bulb mites, Rhizoglyphus spp. (Astigmata: Acaridae), are pests of many crops in the field and in storage. In the field they attack winter grains, potatoes, carrots and sugar beets (Zakhvatkin, 1959; Somermaa, 1972) as well as onion and garlic (Rawlins, 1955). In storage they infest bulbs and tubers of many crops, including gladiolus, hyacinth, lily, narcissus, onions and potatoes (Garman, 1937; Hodson, 1929; Latta, 1939; Sandhu, 1976). The mites also damage seeds of commercial plants (Somermaa, 1972). Damage to onion seedlings in the field resembles "damping off", the plants falling over and withering. Consequently plant stands are thinned. Plants which had withstood the first attack later tip over. Yields are sharply reduced (Rawlins, 1955). Somermaa (1972) reported that grasses are attacked at germination.

In storage, serious losses are incurred. Zakhvatkin (1959) reported that about 50% of all stored onions were destroyed by these mites in the Tartar region of the U.S.S.R. Later Kuznetzov and Tkatchuk (1972) reported from south Russia that 22.2% of all gladiolus corms were damaged by Rhizoglyphus. The mites burrow into bulb scales and into healthy tissue. Bulbs are then attacked by saprophytic bacteria and fungi and consequently rot. Some pathogenic plant bacteria and fungi appear to be associated with Rhizoglyphus when attacking gladiolus. These include Pseudomonas marginata, Fusarium oxysporum f. gladioli and Stromatinia gladioli (Engelhard, 1967; Forsberg, 1959). Transmission by Rhizoglyphus has been demonstrated for P. marginata (Forsberg, 1959), and control of this bacterium was accomplished by applying pesticides against the mite (Forsberg, 1959). Rhizoglyphus was also implicated in bud necrosis, a storage disease of tulips (Munk, 1972) and in dispersal of the plant pathogen Verticillium albo-atrum (Price, 1976). Poe et al. (1979) found pathogens transmitted through the gut of mites to be viable for up to 5 days.

Although mites can survive on rotting plants, they are actively attracted to fresh food, on which they reproduce better (Michael, 1903; Woodring, 1969). Rhizoglyphus rapidly adapts, through alterations in the activity of its digestive enzymes, to changes in its diet (Barabanova, 1976). This considerable adaptation-ability helps mite survival in the soil.

Biological research on Rhizoglyphus has been hindered in the past by the lack of sound systematics. Only recently have Eyndhoven (1968) and Manson (1972) distinguished between the two common species, namely Rhizoglyphus robini Claparede and R. echinopus (Fumouze et Robin). They also began to differentiate between the host plants of these two species. Some taxonomic problems, however, still remain, as Eyndhoven's classification is not universally accepted (Hughes, 1976). These difficulties impede the use of biological data, which may actually pertain to different species. Some of the major contributions in studying the life history of Rhizoglyphus were made by Garman (1937), Kuznetzov and Tkatchuk (1972), Sekiya (1948) and Woodring (1969). More recent publications include Ascerno et al. (1981), Baker (1983) and Muller and Hollinger (1980).

Like many other astigmatic mites, Rhizoglyphus has a heteromorphic second nymphal stage, the hypopus. It is of different structure, being quite sclerotic and lacking mouth parts. The hypopodes' main function is to disperse the species, usually by attaching themselves to insects.

During normal development, the hypopodial stage lasts 1-2 weeks (Garman, 1937), then molting to the third nymphal stage, which resembles the adult. Under adverse conditions the hypopodes may survive for long periods without molting; Hodson (1929) reported that hypopodes kept in glass cells lived 3-4 months, but all died after 5 months. Nothing appears to have been published concerning factors which cause the hypopus to molt, although a high humidity may be postulated. Any attempts at controlling bulb mites must take the resistant hypopus into account.

Various control measures have been applied against Rhizoglyphus. Dipping infested bulbs in hot water, or steam treatment, was recommended but gave variable results (Engelhard, 1967; Garman, 1937). Pesticide dips were also advocated (Ascerno et al., 1981; Engelhard, 1967; Garman, 1937; Kuznetsov and Tkatchuk, 1972; Muller and Holinger, 1980). Other methods of control include application of pesticides in granular form (Forsberg, 1959); fumigation (Hodson, 1929) and broad-spectrum soil pesticides (Jefferson et al., 1956). The effect of many pesticides has, however, been uneven (Engelhard, 1967; Somermaa, 1972), and very few have actually been tried in the field.

Rhizoglyphus has become a field pest of onion and garlic in Israel during recent years. Some growers had complained of large losses, but very little was actually known about this mite in Israel. The present cooperative project was therefore initiated, to study mite biology and control in a comparative way, in Israel as well as the USA, so as to offer some guidelines for its management. However, any efforts at controlling a soil pest are beset by a host of difficulties. Problems to be resolved include establishing an economic threshold, monitoring populations in the soil, establishing the source of infestation, studying the pest's life history and its means of survival during difficult seasons, and trying out control methods. The present project addressed these and other questions, and will offer some guidelines for the control of Rhizoglyphus. The report is divided into three parts (field data, laboratory observations and control efforts) and a general discussion.

2. Field Data

2.1 Methods

2.1.1 Visits and questionnaire

The almost total lack of data regarding the field ecology and phenology of Rhizoglyphus in Israel, the extent of its damage, any factors relevant to mite occurrence and injury, and control methods implemented, necessitated visits to infested fields. In order to standardize the information obtained, a questionnaire was sent to growers and extension personnel. It included questions concerning extent of damage and dates when injury first became apparent, horticultural and plant protection procedures and past field history. Forty questionnaires were duly returned from different parts of the country. Soil samples were obtained during these visits and analysed (2.1.4). Predators were collected whenever encountered, mounted or reared, and sent to specialists for determination. Live Rhizoglyphus and infested plants were regularly brought to the laboratory and examined. Some of these mites were cleared (in Nesbitt's), mounted (in Hoyer's) and examined, Manson's (1972) work being the main authority. Other mites served for rearing.

Data on the occurrence of Rhizoglyphus robini in Virginia were obtained by collecting daffodil bulbs from fields of commercial growers in Gloucester and Matthews Counties, and from a planting at the VPI&SU Horticulture Research Farm in addition to a home garden in Montgomery County. Onions from two home gardens in Montgomery County were also examined. Mites found in the bulbs were preserved in alcohol and later mounted on slides for identification. Some bulbs were put in plastic dishes on a moist plaster of Paris substrate for rearing adults of fungus fly larvae, which were heavily infesting them.

2.1.2 Monitoring Rhizoglyphus in the soil

Being soil dwellers, Rhizoglyphus mites are hard to detect and monitor; a suitable method was thus needed. The tendency of these mites to leave decaying foods for fresh ones (Michael, 1903) was used to devise a "mite trap". A plastic tube (length 135 mm, 24 mm diam.) was fitted with a perforated cap at one end while a wide-mesh metal sieve was welded on at the other. Peeled, wounded garlic pieces were put on the sieve, the tube (Figure 1) then being placed in the soil at a standard depth of 5 cm. A colored ribbon was bound around each tube, to mark it in the field.

Fig. 1: Traps used for monitoring Rhizoglyphus in the soil.

Preliminary results showed that these traps were attracting mites in the soil, but no data are available concerning the area of attraction. The traps, affording a crude estimate of Rhizoglyphus presence in the soil, were then widely used to monitor the mite in various infested fields. A minimum of four traps per field was employed, but as many as 24/field were used in some cases. All fields were visited weekly, the traps and their bait then being changed. The exposed garlic was examined in the laboratory; presence of one or more mites in a trap made it a "positive". The percentages of positives from the total number of traps renewed during each month were then calculated.

Aside from phenological data, the traps also supplied information on the effects of control measures and were used to advise growers concerning mite presence in their fields.

2.1.3 Relationships between mite numbers and damage

Large populations of R. robini were seen on big, healthy plants while low numbers were obtained from heavily injured plants. This suggested that mite-per-plant data might not be appropriate for evaluating the potential of mites to injure crops or for estimating the efficacy of control measures. The matter was explored by comparing parameters from two groups of 50 garlic plants of the local variety, growing in adjacent plots. One group consisted of apparently healthy plants while the others were heavily damaged. All plants were weighed, their length measured and their leaves and roots counted (as indicators of plant condition). Finally mites (excluding eggs) infesting these plants were counted.

A similar comparison was made between two garlic varieties, namely the rather susceptible Ethiopian (which is similar to the local variety)

and the apparently tolerant Brazilian. Corollary observations were conducted on mite distribution on these plants' leaves, bulb and stem plate.

2.1.4 Characterization of Rhizoglyphus-harboring soils

Although soil properties are known to affect the fauna, very little is known about their influence on Rhizoglyphus. Luxton (1981) obtained large numbers of these mites from Danish beech wood soil in which the mull humus fabric had a pH of 6.1-6.9, and the mineral soil, 4.2-7.6. According to Muller and Hollinger (1980), Rhizoglyphus prefers soils rich in organic matter.

Samples were taken from 13 heavily infested fields where garlic or onion was the affected crop. Soil cores (100 cm³) were obtained from several sites in the field, well mixed and analysed. Soil analyses were conducted according to Black (1965) and Richards (1954).

2.2 Results

2.2.1 Visits and questionnaire

The information obtained during visits and extracted from the questionnaire indicated that most injury took place during early winter, i.e., November-December. Grower assessment of damage fluctuated between "light" to "total loss" (a heavily-damaged field is shown in Fig. 2).

Fig. 2: An onion field heavily damaged by Rhizoglyphus robini.

Plants weakened by other factors (drought, weeds, incautious use of herbicides) appeared to suffer more from Rhizoglyphus than plants not stressed. No close relationship could be seen between preceding crops and present injury levels; heavy damage was recorded from plots which

had been left fallow for many years prior to the present affected crop. On the other hand, rather badly-injured fields were seen in the autumn which then recovered by spring, indicating some mechanisms of plant compensation.

Cow or poultry manure, or pellets made therefrom (see 3.1.3) had been applied to over half of the infested fields within two years of mite outbreak. In some plots infestation literally followed the manure spreader (Fig. 3). Rhizoglyphus was also collected on celery roots and gladiolus corms in the field, but no damage to either was seen. Mites were further found on roots of the composite weed Silybum marianum but did not penetrate the live tissue.

Fig. 3: An onion plot treated with manure and heavily damaged by Rhizoglyphus robini (foreground), contrasted with another plot which had not received any manure (background).

Rhizoglyphus spp. occurred in all collections of daffodil and onion from Virginia. Two other genera of mites, not yet identified, were found on the daffodil bulbs from eastern Virginia. The common predatory mite, Hypoaspis (prob. aculeifer Canestrini) was associated with Rhizoglyphus on daffodil bulbs from all three counties.

2.2.2 Monitoring Rhizoglyphus in the soil

The traps were initially used in three infested onion fields, only two of which were irrigated. The higher positive values obtained in April (Table 1) in fields a and b probably reflect the higher prevailing humidities at that season, brought about by irrigation.

Table 1: Trapping Rhizoglyphus in irrigated (a,b) and unirrigated (c) onion fields (TE = total number of examinations; %P = percent of positives).

	<u>a</u>		<u>b</u>		<u>c</u>	
	TE	%P	TE	%P	TE	%P
February	111	36	131	33	48	33
March	87	23	107	22	96	52
April	116	48	156	42	96	28
May	116	15	156	14	30	3

In another onion field a comparison was made amongst a heavily-infested field, a lightly-infested field and a third field which had been treated with Vapam (Metham-Sodium). Although more positives occurred in the heavily-infested plot, mites persisted well in all fields (Table 2).

Table 2: Trapping Rhizoglyphus in heavily-infested (a), lightly-infested (b) and Vapam-treated (c) onion fields (symbols as in Table 1).

	<u>a</u>		<u>b</u>		<u>c</u>	
	TE	%P	TE	%P	TE	%P
February	14	43	14	14	20	40
March	28	75	28	36	40	55
April	28	32	28	36	40	20
May	--	--	--	--	30	3

Traps placed in a field sown to wheat, following a badly mite-damaged onion crop, made it possible to follow mite populations for another season under other conditions. For the sake of comparison traps were also placed in nearby wheat and beet plots with no prior Rhizoglyphus history. Mite populations peaked similarly during February-March (Table 3) with a subsequent spring decline. Mites were thus present in all three plots, although their presence was not manifested by any damage in two of these plots.

Table 3: Trapping Rhizoglyphus in a field previously planted to onion and badly infested by the mite (a) and in wheat (b) and beet fields with no prior mite history (c) (symbols as in Table 1).

	<u>a</u>		<u>b</u>		<u>c</u>	
	<u>TE</u>	<u>%P</u>	<u>TE</u>	<u>%P</u>	<u>TE</u>	<u>%P</u>
December	12	58	--	--	--	--
January	16	50	12	50	6	33
February	16	75	12	33	12	75
March	16	75	12	85	12	58
April	16	25	12	33	12	33
May	14	0	12	0	12	0
June	--	--	3	0	3	0

Traps were also placed in another wheat field formerly used for irrigated cotton. Yellowing, stunted wheat seedlings in this field were infested by Rhizoglyphus. Comparative series of traps were inserted in two adjacent, healthy wheat plots, only one of which was formerly irrigated. No mites were recovered from the drier field. Of the other two plots (Table 4), the injured one had more positives during winter, but not in spring.

Table 4: Trapping Rhizoglyphus in a wheat field in parts injured (a) and uninjured (b) by mites (symbols as in Table 1).

	<u>a</u>		<u>b</u>	
	<u>TE</u>	<u>%P</u>	<u>TE</u>	<u>%P</u>
January-February	20	45	20	5
March	25	40	25	24
April	20	15	20	20
May	15	0	15	0

It thus became evident that Rhizoglyphus is a winter pest of onion, garlic and sometimes wheat in Israel, being rare or inactive during summer. Suspicions that peanut pod injuries may be associated with mites presented an opportunity to monitor for Rhizoglyphus during the summer growing season in an irrigated field. Traps were placed in an appropriate plot prior to sowing in April. No activity was recorded until late May when a break had to be made for intensive horticultural practices. Trapping was resumed by July and continued throughout September. Trapping during these summer months was done at two depths, 5 and 10 cm.

Mites were distinctly more pronounced at the greater depth (Table 5) but occurred at both levels. Irrigation (and possibly the plants' shade on the soil, which would further reduce temperatures and conserve humidity) was thus shown to enable Rhizoglyphus to remain active in the soil throughout summer. No visible damage to peanut plots was observed.

Table 5: Trapping *Rhizoglyphus* at a depth of 5 cm (a) and 10 cm (b) in an irrigated peanut field (symbols as in Table 1).

	<u>a</u>		<u>b</u>	
	<u>TE</u>	<u>%P</u>	<u>TE</u>	<u>%P</u>
July	24	46	24	83
August	18	22	14	36
September	12	42	12	66

2.2.3 Relationship between mite numbers and damage

Injured plants were significantly lighter and smaller than healthy ones (Table 6, a) and carried greater mite loads. These parameters were neither positively nor negatively correlated with actual mite numbers (Table 7). Similar results were obtained upon comparing these parameters between the Brazilian and Ehtiopian varieties. No clear relationship could thus be demonstrated between mite numbers on infested garlic plants and any damage which these mites might cause. This result supplements field observations that dying onion or garlic seedlings may carry not more than four or five mites, and would seriously impede efforts at deciding upon threshold values.

Table 6: Numbers of *Rhizoglyphus* mites and physical parameters of healthy and injured garlic plants of the local variety (a), and a comparison between the Brazilian and Ethiopian varieties (b) (averages per plant in each group).

Variety	Weight (gr)	Length (cm)	No. of leaves	No. of roots	No. of mites
<u>a</u>					
Local, healthy	21.9a	49.7a	7.4a	61.4a	39.3a
Local, injured	4.0b	35.2b	5.0b	26.6b	67.0b
<u>b</u>					
Brazilian	10.7a	35.5a	4.9a	23.7a	80.4a
Ethiopian	3.3b	23.4b	5.0a	13.4b	91.4a

^{ab}Values followed by different letters are significantly different.

Table 7: Correlation values calculated between numbers of mites on healthy and injured garlic plants, and physical parameters of both plant groups.

<u>Plant Condition</u>	<u>Measurement</u>			
	<u>Weight</u>	<u>Length</u>	<u>No. of leaves</u>	<u>No. of roots</u>
Healthy plants	0.0558	-0.1112	0.0082	0.0350
Injured plants	0.2348	0.3568	0.1550	0.1861

Rhizoglyphus inhabited different parts of the Brazilian and Ethiopian varieties. In the former it aggregated mainly on the upper bulb parts and at the leaves' bases, while relatively few mites occurred in the stem plate (Table 8). On Ethiopian garlic (as on the local variety) mites were found mostly in the stem plate and the bulb.

Table 8: Occurrence of Rhizoglyphus on different parts of two garlic varieties (average number of mites per plant).

Variety	Stem plate	Bulb	Leaves	Total
Brazilian	3.7a	30.2a	46.5a	80.4
Ethiopian	29.6b	50.5b	11.3b	91.4

2.2.4 Characterization of Rhizoglyphus-harboring soils

The great variability found in the various results of soil analysis in Israel (Table 9) probably reflects on the mite's diverse habitats and versatility. It is the only striking result obtained. A few data will be discussed.

Soil texture is determined by the ratios between clay, silt and sand, and affects the water holding and aeration properties of the soil. Rhizoglyphus was found in loam to clay soils, but never in sandy ones.

Organic material: the values obtained varied between 0.19 and 1.63%, which are rather low. Bearing in mind that Muller and Hollinger (1980) considered Rhizoglyphus to prefer organic soils, these mites appear to thrive under a great variety of soil conditions.

Conductivity: this property is affected by and directly proportional to the concentration of dissolved anions and cations in the soil, as well as being indicative of the osmotic pressure in the soil solution. Both parameters affect the mite by influencing the humidity available to it. They also affect commercial and weed plants, and the soil microorganisms.

Acidity: soils in Israel usually show a basic soil reaction, and all samples varied only between pH 7.8-8.1. This reemphasizes the great versatility of Rhizoglyphus, as Astigmatic mites are believed to prefer acidic soils (Edwards and Lofty, 1974).

Mineral content: the large variability found here might be explained in the terms used in regard to acidity.

2.3 Miscellaneous

The occurrence (sometimes to pestiferous levels) of Rhizoglyphus in fields which had remained uncultivated for long periods raised the question of the mites' origin. Two possibilities were investigated: that mites were introduced with the seed (or clove) material, or that

Table 9: Analysis of soils from 13 onion or garlic fields infested by Rhizoglyphus robini

Analysis	No. Field												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Texture	Loam	Loam	Loam	Loam	Loam	Loam	Loam	Clay	Clay	Clay	Clay	Clay	Clay
Organic material (%)	1.05	1.24	1.12	1.25	1.12	1.16	0.91	0.97	1.17	0.96	1.13	1.63	1.53
Water in saturated past (%)	46.1	47.6	44.9	57.1	55.1	57.5	52.8	65.2	64.1	60.4	61.1	68.5	73.5
Conductivity (semens=mmhos/cm)	0.60	0.78	1.10	1.67	1.80	0.80	1.62	2.26	0.71	0.70	0.53	0.92	1.54
pH	8.1	8.1	8.1	8.0	8.0	8.2	8.0	7.9	7.9	8.0	8.0	7.8	7.8
NO (g/g)	40.0	40.0	77.0	69.0	165.	47.0	37.0	137.0	30.0	26.0	24.0	118.0	226.0
P (g/g)	48.5	60.0	55.0	25.0	20.0	30.0	26.5	12.0	31.0	11.0	26.0	26.0	20.0
K (milliequivalent/litre)	0.20	0.25	0.53	0.31	0.27	0.25	0.12	0.25	0.15	0.10	0.19	0.15	0.31
Cl (milliequivalent/litre)	1.0	1.5	3.3	7.4	9.5	1.2	5.0	10.2	3.5	4.2	3.3	4.1	3.2
Calcium carbonate (%)	14.2	16.9	17.3	20.9	22.2	13.3	15.7	19.6	13.1	16.3	12.2	10.6	14.3
Ca+Mg (milliequivalent/litre)	4.6	5.6	7.8	9.8	10.2	6.0	10.8	17.5	5.0	4.6	3.2	6.2	9.4
Na (milliequivalent/litre)	4.5	4.8	6.25	5.9	5.9	4.8	7.5	5.2	2.4	1.9	1.75	2.4	3.7
Salinity Index	3.0	2.9	3.16	2.7	2.6	2.8	3.2	1.8	1.5	1.3	1.4	1.4	1.7
B (g/g)	0.77	0.76	0.81	0.36	0.37	0.56	0.64	0.17	0.69	0.49	0.60	0.59	0.69
Clay (%)	29.5	29.5	28.3	39.0	37.8	39.5	37.8	45.3	52.0	44.5	47.0	47.0	52.0
Sief (%)	35.0	42.5	43.7	41.3	42.5	40.0	35.0	40.0	37.5	37.5	35.0	37.5	35.0
Sand (%)	35.5	28.0	28.0	19.0	19.7	20.5	27.2	14.7	10.5	18.0	18.0	15.5	13.0

they had come in with the manure (with which they often seem to be associated).

Onion is grown from seeds too small and dry for mites. Garlic, however, is grown from cloves which are dry-stored from the preceding year. Samples of compound garlic bulbs were obtained from storage and examined throughout the summer. Dead, shrivelled mites were sometimes found between the cloves, but no live ones were seen among the hundreds of bulbs examined. Therefore it is rather unlikely that the heavy mite outbreaks can be attributed to infested seed (clove) material. The other possibility, manure, was examined by running cow and poultry manure from infested fields through modified Tullgren funnels, or by processing such samples by flotation techniques. Although a large number of mite species was thus obtained, no Rhizoglyphus were found.

2.4 Discussion

The data presented above allow us to offer an outline of the phenology of Rhizoglyphus in Israel.

During summer the soil in unirrigated areas is too hot and dry for the mites, which either perish or move to deeper strata. As temperatures drop in autumn and soil cultivation commences for winter crops, mites may return to upper layers. Irrigation further reduces soil temperatures, and may facilitate that movement. The abundant plant material (commercial crops, weeds, fungi) which then grows serves as nutrition for the mites. Addition of manure, allows even larger mite populations to develop.

Upon planting onion or garlic, a new, apparently very attractive nutrient source is added, as shown by the trapping data. Rhizoglyphus feeds on the young, small roots and at the stem plate, causing economic damage.

Later, in winter, temperatures apparently drop below the mites' threshold, stopping or slowing down their life processes. Plant growth, however, continues, and the onions and garlics gain in strength. They are thus less susceptible to renewed mite attack in early spring. The cycle then repeats itself.

In irrigated plots the mite continues its activity throughout summer, feeding on non-commercial plants or causing little injury to commercial ones. It does not appear to be a pest of flower bulbs and corms in the field in Israel.

In contrast to its role in Israel, Rhizoglyphus robini is an important pest of flower bulbs (lily) and corms (gladiolus) in the USA. Damage is greatest in the areas where large commercial plantings occur, such as Florida, California, and Oregon.

The failure to find correlations between mite numbers and measureable indices of injury suggests that establishing time of attack may be more important than quantitative data on Rhizoglyphus

populations. Small numbers infesting seedlings in the autumn are more injurious than large numbers in spring. The traps could, as has been shown, be useful for early monitoring.

The relative tolerance of the Brazilian compared to the Ethiopian (and local) varieties may well depend on the different settling sites of the mites on these plants. It may be a promising lead for plant breeders.

Mite damage to wheat appears to be quite rare. However, its actual occurrence suggests that it may recur in other wheat plots.

The occurrence of Rhizoglyphus spp. in two very different climatic and soil types in Virginia also suggests that it has a wide tolerance of habitat conditions. Collections were made in March from counties in eastern Virginia, where the soil is sandy and usually stays quite moist. Soil temperature was at or below 5°C at an elevation of about 1800 m with a silt-loam soil subject to periodic drought conditions. At the time of collection in January soil temperatures average 0°C.

3. Laboratory studies

3.1 Methods

3.1.1 Rearing

Colonies of Rhizoglyphus robini were collected from infested garlic bulbs; they were mass-reared by two methods.

A. Leguminous seeds (mainly peanuts) were soaked in tap water and placed in petri dishes (9 cm diam.) padded with filter paper (Whatman No. 1). Mites were transferred thereon and rapidly developed large colonies under room or 24°C conditions. Hypopodes (mites of the second, heteromorphic nymphal stage) were found in these cultures when they began to dry up).

B. An artificial diet, based on a caterpillar rearing medium (Bot and Meyer, 1967). It consisted of alfalfa meal (50 gr), potato meal (12.5 gr), agar (10 gr), Nipagin (1.2 gr), ascorbic acid (2 gr), cyntomycetin (0.125 gr) and chlorine chlorate (9.0 ml). The solid ingredients were mixed with 150 ml water, the agar then being added and stirred along with another 100 ml water. The medium was then autoclaved, poured into petri dishes and cold-stored. Several thousand mites were grown in each dish, which held for several months. The main drawback to this method was difficulties in mite recovery from the medium, into which they burrowed. It was therefore used mainly for stock maintenance. When even-aged cultures were required, peanut seeds were placed on top of this medium for some hours (Fig. 4) and, once covered by mites, removed to petri dishes. Very few hypopodes developed on this medium.

In Virginia, stock cultures of Rhizoglyphus robini were started from mite cultures which had been maintained in the laboratory for several years, originating in Oregon. The standard rearing chamber was a round plastic dish with a friction lid, 5 cm in diameter x 3.7 cm deep. It was one-third filled with a mixture of 1 part powdered charcoal to 100 parts plaster of Paris. Two sources of food were tried, both of which were successful: powdered active dry yeast and the artificial diet described under Section 3.1.1B.

Fig. 4: Rhizoglyphus infesting peanut seeds from the artificial medium.

3.1.2 Observation units

Many observations were conducted on mite behavior in petri dishes padded with filter paper.

Special plexiglass cages were devised and constructed for observing individual mites. Plexiglass sheets (4 mm thick) were cut to rectangular pieces (7.5 x 3.5 cm), into which three holes (each 1.2 cm diam.) were drilled. Bits of black filter paper (Eaton Dikeman, Mt. Holly Springs, Pennsylvania, Grade 8613), measuring 2.0 x 1.5 cm, were placed on top of the holes. A small depression was then made in the paper on top of each hole, food introduced and a mite placed therein (Fig. 5). The paper was covered by a microscope slide and held in place by two metal clips. Each rectangular plexiglass piece thus served as three individual observation cages. The whitish mite eggs, immatures

and adults can easily be seen through the glass slide against the dark filter paper background. Such cages may conveniently be placed in controlled-humidity desiccators.

Fig. 5: Plexiglass cage for observing individual mites. Above: general view; below: longitudinal cut showing depressions in filter paper and a mite in situ.

Observation units for tests done in Virginia were standard plaster of Paris dishes or petri dishes.

3.1.3 Diets

Many diets were offered to Rhizoglyphus in these experiments. When assessing the development and fecundity of individual mites, peanut seeds (var. Virginia), garlic cloves (local var.) or filter paper (Eaton Dikeman, as above) were used. Peanut seeds represented a diet rich in protein and fat (27 and 44%, respectively, according to Guggenheim et al., 1973). Garlic cloves contain far less protein and fat (5 and 0.2%, respectively) as well as being a crop which is damaged by the mite in the field. Wetted filter paper may enable the mite a rather limited rate of development. Seeds and cloves were offered as finely-cut fragments which were replenished at least twice a week.

Other diets offered included: (1) Peanut extract (seeds soaked in 1.5 their volume in tap water for two days, filtered and diluted to the required concentration).

(2) Poultry and cow manure pellets (Zewel, product of Shaham, Givat Ada, Israel). Their chemical composition (obtained from the producers) is presented in Table 10. Pellets were offered either as wetted solids or as a water extract (50 gr dry/200 ml tap water), soaked onto filter paper.

Table 10: Chemical components (dry weight) of two types of manure pellets.

	Cow manure pellets	Poultry manure pellets
Organic material	57.0%	65.0%
N	2.5%	3.5%
P ₂ O ₅	2.4%	2.4%
K ₂ O	3.0% 80%	2.8% 89.4%
Ca	2.04% only	2.81%
Mg	0.77%	0.88%
Fe	0.17%	0.06%
Zn	125 ppm	358 ppm
Mn	167 ppm	362 ppm
Cu	50 ppm	50 ppm

(3) Garden N:K:P fertilizer (10 gr/100 ml water) (Haifa Chemicals Ltd, Israel), soaked onto filter paper. Other fertilizers (NSO₃, KCl) were similarly prepared and offered.

(4) Fresh cow and poultry manure, collected in the field and presented, either intact or as extracts on filter paper in petri dishes (Gerson, 1983).

(5) Soil from an infested field was collected and pressed, the affluent liquid being soaked onto filter paper. The same procedure was repeated for soil dug out from under cow and poultry heaps in the field.

(6) Dried oat and vetch hay, and barley straw were collected in the field, washed and put in padded petri dishes.

(7) Roots of Silybum marianum (Compositae) and Malva sylvestris (Malvaceae) were obtained from infested fields, washed and offered as above.

(8) Various soil arthropods (Table 14) were offered freshly-macerated in padded petri dishes.

Studies in Virginia on the influence of various diets focused on the population growth of mites on potato dextrose agar (PDA) and PDA plus one of the following five soil fungi: Fusarium oxysporum f. perniciosum, Botrytis gladiolorum, Curvularia trifolii f. gladioli, Rhizoctonia solani, and Stromatinia gladioli.

3.1.4 Experimental procedures

Life history experiments were initiated with 30-40 eggs of uniform age. Saturation humidity was maintained throughout. Development, fecundity and longevity on garlic were assayed at 16, 22, 27 and 35°C; on peanuts and filter paper only at 27°C.

Single females were isolated, upon eclosion, in cages with only one male; each couple remained together throughout their lives. Egg hatch, immature and adult survival, duration of all stages and fecundity were recorded daily.

The suitability of the other diets and their effect on mite survival and fecundity were assayed by two complementary methods. In the first, ten Rhizoglyphus pairs were placed on each food (usually in two or four replicates) and an estimate was obtained by periodical, visual examinations of the colony. Presence of immatures, rate of mating (see below) and the state of the diet were all taken into account. During this phase of the study it was noted that mites kept on richer diets tended to mate more frequently than those on poor foods. A method for estimating food quality, based on mating frequency, was therefore developed.

When mating, the male of Rhizoglyphus places the posterior end of its hysterosoma above that of the female, so that their mouth parts point in opposite directions. Once united, the two may move about, the male's hysterosoma remaining above that of the female; their appearance then is quite unmistakable (Fig. 6). Mating is a prerequisite for oviposition, as isolated virgin females never produce any eggs.

Fig. 6: Male and female of Rhizoglyphus robini in characteristic mating position (setae omitted).

A method based on observations taken once a day must take into account the duration and frequency of the observed phenomenon. A total of fifty couples of R. robini, seen in copula, were individually placed in the plexiglas cages for one night at 22°C. During the following day all couples were checked and recorded for mating or non-mating every

half-hour, for 9 1/2 continuous hours (a total of 19 observations). Between checks the cages were kept under high humidity conditions in the same room, where temperatures fluctuated between 21 and 22.5°C.

Only 2 (4%) of the couples failed to pair during the entire observational period (Fig. 7), while others were seen together for over 2 h. Most couples broke off once or more and then resumed pairing. Altogether 219 mating observations were recorded. Average mating time was slightly over 2 h per couple (calculating all single observations as a half-hour pairing), and a mean of 11.5 couples were together at any time during the experimental period. Kuznetsov and Tkatchuk (1972) had reported that the mating of R. echinopus lasts for about 30 min.

Although no corroborative data are available, such prolonged, on-and-off-and-on again matings during a single day of adult life are strongly suggestive of multiple inseminations.

Fig. 7: Mating frequency of 50 couples of Rhizoglyphus robini observed every half-hour for a 9 1/2 h period. Every square represents a couple mating at the registered hour.

Peanut seed extract was diluted (with tap water) to obtain 10, 20, 40, 60, 80 and 100% (no dilution) concentrations. Two millimeters of every concentration were then put in each of 5 padded petri dishes. Ten couples of R. robini, of undetermined age, were added to each of the 30 dishes, and all were maintained at $22 \pm 1^\circ\text{C}$. A few drops of tap water were added as required. Daily counts of mating couples were conducted for 23 days. This 23 day span is about one-and-a-third times (1 1/3) the cycle of first progeny to first progeny of R. robini on that food at 22°C (see below), a period which provides a reasonably accurate estimate of generation time (Wyatt and White, 1977). All adults were counted at the end of the experiments. This trial was conducted with 6 concentrations of the same food, in order to reduce possible experimental errors which might have been introduced by using diets of various origins. To supplement these data another test was run with

three different foods. These were peanut extract, poultry manure extract and cow manure extract (all 100%). The experimental procedure employed was quite similar, except that only 5 couples were placed in each of 4 dishes per extract.

During the first five days there were no discernible differences in number of couples mating between the various treatments. By the end of the second week, however, four groups could be characterized, and these remained distinguishable until the experiment's end. The largest number of pairings was seen amongst mites offered the undiluted peanut extract (Fig. 8). At the three lower concentrations (80, 60 and 40%), they

Fig. 8: Average number of matings of *Rhizoglyphus robini* observed on the various peanut extracts during 23 days together with the mean number of adult mites obtained from these foods. Bars on tops of columns represent the 95% fiducial limits (± 2 SE).

coupled at a lesser but similar rate (except for an unexplained rise at 40%). The two poorer foods (20 and 10%) reduced the matings even more, a significant difference being obtained between these values. The numbers of adults obtained from the various peanut concentrations were grouped in four distinct groups which paralleled the values of the observed matings (Fig. 8). The numbers of matings and adults from each

dish were paired, and their overall correlation calculated. When the number of adults was used as the independent variable and that of matings as the dependent one, a slope of 0.752 was obtained, the intercept being at -21.965. Upon making matings the independent variable, adults the dependent one, the slope was 1.040 and the intercept was at 89.144. Spearman's r was 0.884 ($P < 0.01$) in either case.

The mean number of matings seen on peanut extract was 157.0 ± 84.7 (SD) per dish, with an average of 253 ± 69.7 adults per dish. The corresponding values for poultry manure extract were 86.0 ± 32.6 and 111.2 ± 46.0 and for cow-manure extract 2.6 ± 1.7 and 5.0 ± 1.8 . Foods of different origin likewise induced parallel mating and adult values.

The data summarized in Fig. 7 showed that a fifth to a quarter of all mites were mating at any given time under the particular experimental conditions; a definable proportion of the population may thus be seen in copula during the usual hours of the day. Some mating periods shown in Fig. 7 were considerably longer than was formerly recorded. The results presented in Fig. 8 demonstrate that food quality may be evaluated by counting the numbers of Rhizoglyphus mating during a certain period. The $1 \frac{1}{3}$ d value defined above appeared to provide a reasonable estimate of this period. The need for the additional third of the first-progeny-to-first-progeny period ensued from the similar, low number of matings observed during the experiment's first week. Differences in copulation frequency between mites on the various extract concentrations became apparent only towards the end of the second week. Food concentration (and possibly also quality) does not therefore immediately affect the number of mites mating. This conclusion is supported by many observations that even starving Rhizoglyphus sometimes copulate. By the third week two additional, food-mediated factors came into play. The first was the mite's more rapid development on the better foods, which caused its first progeny to begin mating around day 17. The other factor was the larger number of eggs produced on the better foods, the 100% concentration giving rise to the largest number of progeny. To sum up, the superior foods induced more copulations, caused Rhizoglyphus to produce more progeny, and shortened their developmental time. The compounded effect of these factors engendered the higher mating frequency observed on the concentrated extracts. The fewer pairings noted on the more diluted diets resulted from the same factors, compounded in the opposite direction.

The close relationship obtained between number of couples pairing during the experiment and number of adults counted at its termination, and the similar separation of each set of values into four groups (Fig. 8) shows that both methods will supply similar results. In other words, determining mating frequency is as good a method for evaluating food quality as counting the resultant progeny. The period during which matings should be counted could be shortened, although with a consequent rise in variation. Upon using only the couplings observed in the final 6 days of the experiment (the extra third of the $1 \frac{1}{3}$ d), the following values were noted: on 100% extract, 374 ± 80 matings; on 80%, 130 ± 40 ; 60%, 116 ± 16 ; 40%, 187 ± 46 ; 20%, 62 ± 29 ; and 10% 4 ± 2 . Despite the

unexplained high value at 40%, the overall pattern persisted. The same pattern was also obtained when the mites fed on the manure extracts. We concluded that mating frequency of R. robini is directly dependent on its food; and subsequently used the former to estimate the latter.

The occurrence of hypopodes in the peanut seed cultures made it possible to observe the effect of various humidities and temperatures on their molting. Hypopodes (30-in 6 cages-for each humidity-temperature combination) were kept in desiccators maintaining constant relative humidities. These (87, 90, 93, 96 and 100 % r.h.) were obtained with sulphuric acid or with various salts, prepared according to Solomon (1951) and Winston and Bates (1960). Free water (distilled) was also used. Desiccators were held at 16, 24, 27 and 35°C, in complete darkness. All cages were examined daily at the same hour. Numbers of molted mites and the period required for this process were then recorded. All tritonymphs obtained at 27°C and 100 % r.h. were taken out, placed on peanut seeds in a separate dish and reared until maturity, to determine the sex ratio.

One of the main characters separating R. robini from R. echinopus is the length of the internal scapular (sc i) setae. Eyndhoven (1968, 1972) and Manson (1972) maintain that R. echinopus has relatively long setae, while Hughes (1976) believes that it actually lacks sc i, calling the species with long sc i setae R. callae Oudemans. It thus appears that the presence/absence of these setae and their lengths are of taxonomic importance.

During the life history studies it was observed that mites reared on different diets differed in body size. The possibility that length of the sc i setae of R. robini is a variable, nutrient-dependent character was explored by separate rearings. A single female was picked at random from a population kept on peanut seeds and isolated for 24 hours in a cage. The thirty-odd eggs deposited were separated into three batches and each placed on a different diet in petri dishes. Diets assayed were: water-soaked peanut seeds, water extract of peanut seeds and filter paper wetted by tap water. The dishes were sealed with parafilm strips (to prevent mite entry or escape), which were changed once a week while adding water. All cultures were kept in the laboratory (22 ± 3°C) and total darkness for five months. Large populations developed on peanut seeds and extract, but only a very small one on filter paper. At least 10 males were taken from each culture, cleaned, mounted and examined. The body (from tip of chelicerae to tip of opisthosoma) and leg I (from tip of tarsus to base of femur) of each specimen were then measured, along with the external scapular (sc e) and sc i setae. Leg and setal measurements from the two sides of the body were averaged.

Males reared on peanuts were glued onto stubs, coated with palladium and scanned at 25 KV with a JEOL scanning electron microscope (model JSM-35C).

Various ancillary observations and a method of separating Rhizoglyphus from plant tissue by enzymatic means will separately be described.

For studies on population growth and hypopus production in relation to soil fungi, the general procedure was to start with adult mites on agar, with or without a fungus present, and let the population develop for four weeks. After this time numbers of mites in various stages were recorded. Two experiments were done.

The standard medium for the two tests in the first experiment was potato dextrose agar (Difco), 39g/l, to which Chloramphenicol and trace elements were added. Fungal cultures were started by cutting two plugs from a one-week-old culture of the particular fungus with a no. 6 cork borer and placing them on 15 ml of PDA in a plastic petri dish. Fungi used were: Fusarium oxysporum f. perniciosum, Botrytis gladiolorum, Curvularia trifolii f. gladioli, Rhizoctonia solani, and Stromatinia gladioli. After four days in the dark at 26°C each dish (four replicates per fungus) was infested with 5 female and 5 male R. robini from an even-aged culture (ca three weeks old) reared on dry yeast. All four dishes in a particular treatment were put in glass peanut butter jars to retain moisture and restrain mite movement among treatments. After 28 days in the dark at 26°C counts were made of eggs, hypopodes, other juveniles, adult females and adult males.

In the second experiment the nutritional quality of PDA was tested by using half as much PDA powder to make the agar and comparing population growth of mites on this agar with that on the regular concentration. A culture of Curvularia trifolii f. gladioli grown on the half concentration agar was tried also. Four replicates of each agar were inoculated with 5 female and 2 male mites and the population allowed to grow for 4 weeks.

In addition to population growth of Rhizoglyphus robini in relation to soil fungi, a food preference test was conducted to determine if this mite could locate and would prefer to feed on bulbs infected with Fusarium.

A daffodil plant (variety Mt. Hood) was dug on April 22. There was no bloom on this plant although many other daffodils were blooming. The bulb was 23 mm wide and 26 mm high. It was cut into quarters and the innermost scale used for the test. A no. 1 cork borer sterilized in 100% ETOH was used to cut discs of daffodil tissue. Four discs were infected with Fusarium oxysporum f. perniciosum by placing them for 48 hours at 26°C in the dark on a one-week-old culture of the fungus growing on PDA agar. Uninfected discs were cut on the day the test was begun from the same daffodil bulb, which had been held in the refrigerator. One disc of each type was placed in a 1/2 dram shell vial, and the two vials were put opposite each other in a 9 x 1.5 cm glass petri dish which had two layers of brown paper toweling covering the bottom. The toweling was moistened with distilled water and a group of mites (including at least 30 adults) of mixed age distribution and stages was transferred from stock cultures fed on yeast to the center of the petri dish. The glass petri dishes were placed inside a 15 cm diameter x 11 cm deep plastic container with a tight fitting lid to reduce moisture loss. Each vial was examined after 24 hours in the dark at 26°C for the presence of mites.

Three kinds of tests were done to determine the effect of various foods on transformation of one week old hypopodes. Petri dishes were used in tests of transformation on fungi and the standard plaster of Paris dishes were used for the remaining tests (Section 3.1.1). The temperature in the room was $25 \pm 4^{\circ}\text{C}$. In the first test the effect of different quantities of dry yeast (the food on which the mites had been reared) on transformation was examined, first using an abundant amount of yeast (20 grains) vs. a little (4 grains). The data were inconclusive so the experiment was repeated using 0, 1, 3 and 9 grains of yeast. In both cases the number of hypopodes that transformed in four replicates of 15 each was recorded every other day for a week. In a second test transformation in the presence of yeast was compared to that in the presence of Curvularia trifolii f. gladioli. The test cell was a cork-borer cut plug of PDA from a one-week-old culture of the fungus placed on the plaster of Paris. Observations were made of the number of hypopodes in four replicates of 40 each that transformed on alternate days for 30 days. The third test was to determine transformation of hypopodes when they were directly on a fungal culture. PDA dishes were prepared with four-day-old growths of Fusarium oxysporum f. perniciosum, Botrytis gladiolorum and Curvularia trifolii f. gladioli. Transformation was recorded on alternate days over a nine day period for the four replicates of 15 hypopodes each.

A final test was designed to see how two different temperatures affected hypopus survival and transformation. To test survival of hypopodes under low temperatures, four standard plaster of Paris containers were prepared with 15 hypopodes each taken from a stock culture of Rhizoglyphus robini reared on dry yeast. Only distilled water (no food) was added to the dishes. The four dishes were placed inside a 15 cm diameter x 11 cm deep plastic container with a tight fitting lid to retain moisture. The large container was left in a refrigerator (4°C) for almost 6 months. Upon removal from the refrigerator, the container was held for three days at room temperature (ca 27°C) before the number of transformed hypopodes was counted.

Survival of hypopodes at 26°C was tested in the same way by placing plastic dishes in a controlled temperature chamber. The number of tritonymphs (transformed hypopodes) was counted twice, at the end of one and two months.

3.1.5 Computations

Survival of immatures was calculated as suggested by Varley et al. (1973). The effect of temperatures on development was computed by using the equilateral hyperbola equation, which assumes that the product of development time and temperature above a certain threshold is a constant (the so-called "thermal constant," or ThC, Bodenheimer, 1958). The parameters of the equilateral hyperbola (ThC and C, the developmental threshold) are derived from the equations $\text{ThC} = 1/b$; $C = -a/b$, a and b being coefficients obtained from the regression of developmental rate on temperature. Life tables were constructed and calculated according to Birch (1948) and Watson (1964); the simplified method of Wyatt and White

(1977) was also tried. The latter was based on the observation that about 95% of the progeny of certain short-lived arthropods (aphids and tetranychid mites) were produced during the first few days of reproduction, usually in about 2d (d being number of days from birth to birth of first offspring). Number of progeny born during the 2d period (M_d) was the product of age-specific survival, l_x , and age-specific fecundity, m_x ($m_d = \sum l_x m_x$). Intrinsic rate of increase may now be obtained from the expression $r_m = c(\log M_d/d)$, where c is a correcting factor. From their computations Wyatt & White (1977) concluded that $c = 0.74$ was appropriate for the arthropods noted above. They further showed that generation time, T , may be calculated from d/c , or $d/0.74$.

Data from work done in Virginia were analyzed using an analysis of variance followed by Duncan's Multiple Range Test, except for results from the food preference test. These percentages were analyzed using the Normal Approximation to the Binomial Distribution for One Sample.

3.2 Results

3.2.1 Life history and life tables

3.2.1.1 Immature states

Development rate of Rhizoglyphus was accelerated from 16°C through 22°C to 27°C (Table 11), but declined at 35°C, indicating that this temperature is beyond the mite's optimum. Sekiya (1948) has already shown that the development of R. echinopus was retarded at 33°C. Mites offered peanuts or garlic required about the same period to mature at 27°C (the former food imparting a slight advantage), but almost four times as many days on filter paper (at the same temperature). The stages which suffered most mortality on filter paper, and on garlic at 16°C, were the larvae and protonymphs, while at 35°C these were again the larvae, and the eggs. At 22°C and 27°C, on the other hand, mortality was low and uniform during all stages, on peanuts as well as on garlic. The high, similar "adjusted survival" rates obtained under these circumstances (last column of Table 11) indicate that the latter were close to the mites' optimal conditions.

The calculated threshold of development (obtained from the regression of developmental rate on temperature, Fig. 9) was at 11.8°C, and 184.8 day-degrees were required to reach adulthood. Data recorded at 35°C were not used in this calculation.

Besides affecting rate of development (Table 11), the various nutrients also greatly influenced the dimensions of individuals which had fed on them. Mites given peanuts attained, at maturity, almost twice the size of those offered filter paper; individuals on garlic were intermediate in this respect.

3.2.1.2 Oviposition and longevity

Mating (at 27°C) usually occurred as soon as adults had begun to feed, and often recurred. Females on peanuts began to oviposit one day after mating, those on garlic waited another day. Rate of oviposition

Table 11. Duration of development (in days) and a survival of immature stages of Rhizoglyphus robini at four temperatures on three different diets. Numbers in "Total" column denote mites which matured, while values in parentheses signify number of individuals observed.

Food	Temperature (.C)	Eggs	Larvae	Protonymphs	Tritonymphs	Total	Adjusted survival (LX)
Garlic	16	10.7 (40)	13.0 (32)	7.3 (24)	9.2 (21)	41.0 (10)	40.2 (9)
	22	5.7 (42)	6.5 (42)	4.6 (39)	4.4 (35)	21.6 (13)	19.2 (22)
	27	3.6 (38)	3.0 (38)	2.8 (38)	2.6 (38)	11.6 (21)	12.6 (17)
	35	3.4 (36)	3.5 (29)	4.2 (17)	4.1 (16)	15.2 (13)	0.360
Peanuts	27	3.3 (32)	2.6 (32)	2.4 (32)	2.1 (32)	10.1 (18)	11.1 (14)
Filter paper	27	3.5 (25)	9.5 (23)	15.4 (16)	10.7 (10)	39.1 (7)	0.279

Fig. 9. Effect of temperature on the developmental rate and time to maturity of *Rhizoglyphus robini* reared on garlic.

rose very rapidly thereafter, peaking at about 40 eggs/female on the seventh day for peanut-feeding mites, and at about 20 eggs/female, on the tenth day, for those offered garlic. Mean fecundity was 690 eggs/female on peanuts (range 277 - 935, the maximum deposited by a single female in one day being 53 eggs), close to 400 eggs/female on garlic (range 137 - 555, maximum for a single female in a single day, 33). Females kept on both diets oviposited during a similar period (Fig. 10). At 16°C oviposition began on the fifth day of adult life, its rate peaking during the second week (Fig. 11). Mean fecundity was 133 eggs/female, and the recorded maximum for a single female was 568. Some females, however, produced no eggs at all, and others oviposited less than a dozen. No eggs were deposited by females which had developed on filter paper, with two exceptions. The males which had been placed with the latter females were found dead after one day, possibly killed by their mates. Each of these females subsequently deposited a single egg. This suggests that a minimal measure of fertility had been retained by these mites, notwithstanding their development under conditions of virtual starvation. Females raised and held at 35°C produced no eggs, indicating that they and/or the males were rendered infertile by the high temperature.

Total longevity of the two sexes was quite different. At 27°C, females on peanuts lived approximately 40 days, ovipositing for most of this period. The comparative males, remaining alone in the cages, survived an average of 73 days. On garlic the values were 31 and 62 days, respectively, and at 16°C some males remained alive for several months. On filter paper (27°C) and at 35°C (on garlic) adult longevity reached only 15.1 and 14.3 days, respectively.

Counts of laboratory-reared adults indicated that their sex ratio was 1:1; this is similar to the rate recorded for R. echinopus (Woodring, 1969).

3.2.1.3 Life tables

Life tables were constructed for mites maintained at 27°C and 16°C (Figs. 10 and 11). The net reproduction rate (R_0), finite rate of increase (λ), intrinsic rate of increase (r_m) and mean generation time (T) were calculated by the conventional method; the latter two values were also computed according to the simplified procedure (Table 12).

3.2.2 Effect of various diets on mite increase

3.2.2.1 Observations

As noted (2.2.1), Rhizoglyphus often occurred in the field where cow or poultry manure had been applied. When mites were placed on solid bits of either manure they developed quite well and large populations grew there within two-three months. Feeding was often mainly on the filter paper, which had become impregnated with manure extract. However, whenever the paper began to dry up, the mites aggregated on, in and under the remaining manure. After three months the cow manure started to lose its original shape (Fig. 12).

Fig. 10. Age-specific survival (l_x , triangles) and age-specific fecundity (m_x , circles) of *Rhizoglyphus robini* maintained on two diets (filled-in symbols = peanuts; empty symbols = garlic) at 27°C.

Fig. 11. Age-specific survival (l_x , triangles) and age-specific fecundity (m_x , circles) of *Rhizoglyphus robini* maintained on garlic at 16°C.

Table 12. Net reproduction rate (R), intrinsic rate of increase (r), finite rate of increase (λ), mean generation time (in days) (T), number of days from birth to first birth (d) and effective number of progeny by day d (Md) of *Rhizoglyphus robini* maintained at two temperatures on two diets. Values a - d calculated according to Birch (1948) and Watson (1964), values e - i according to Wyatt & White (1977).

Temperature	Diet	(a) R	(b) r	(c)	(d) T	(e) d	(f) M	(g) r	(h) Percent of deviation (b - g)	(i) T	(j) Percent of deviation (d - i)
16 C	Garlic	36.79	0.064	1.10	56.33	45	35.48	0.059	7.8	60.8	7.6
27 C	Garlic	134.47	0.218	1.24	22.48	15	82.26	0.218	--	20.3	9.7
27 C	Peanuts	257.63	0.285	1.33	19.48	12	125.40	0.298	4.6	16.2	16.8

Fig. 12: Cow manure after three months' exposure to Rhizoglyphus. Note fluffy filter paper, where mites had fed on the manure extracts.

Another photograph, taken three months later (Fig. 13), shows that the manure had lost its original shape, with mites still feeding on it. This probably reflects manure breakdown in the field.

Fig. 13: Cow manure after six months' exposure to Rhizoglyphus. Small white dots on the manure are mites.

Rather similar results were obtained with the manure pellets. These tended to disintegrate when wetted; Rhizoglyphus then moved in to feed there and below, on the leached extracts. Large (several thousand strong) populations developed on these pellets within a relatively short time. Few mites, however, actually burrowed into the manure or pellets.

Observations on mites on NPK-impregnated filter paper showed little feeding in the beginning. Later the dishes became overgrown by molds on which the mites fed. No trace of the molds remained after three months and the mites consumed the filter paper, becoming quite numerous.

Observations were made on Rhizoglyphus feeding on hay and straw. Initially mites ate molds which had developed there, but later they began to burrow into both. Rhizoglyphus populations were on the increase when, after 60 days, the observations had to be terminated.

3.2.2.2 Experiments

For technical reasons experiments were run in two sets: the first with five mite pairs/dish, in five dishes, for 23 days, and the second with ten pairs/dish, for 50 days. It was evident that mites survived well on fresh and pelletized manure (Table 13) (the larger numbers obtained from poultry manure probably reflected the higher nutrient content of this diet, Table 10). Soil and mineral extracts allowed very little to no reproduction, and this experiment on NPK extract produced quite different results from the one described earlier. The difference may be attributed to the presence of molds in the latter experiment. Peanut extract was included only for comparative purposes, and the good association between mating values and number of adults obtained adds credence to the former index (see also Table 14).

Table 13: Matings and adults of Rhizoglyphus robini obtained after 23 days on extracts of various potential diets.

<u>Extract of</u>	<u>No. matings</u>	<u>No. adults</u>
Peanuts	157	253
Cow manure (fresh)	3	5
Cow manure (pellets)	31	42
Poultry manure (fresh)	86	111
Poultry manure (pellets)	34	46
Soil from infested field	2	3
Soil under cow manure	0	1
Soil under poultry manure	1	4
Fertilizers: NPK	0	0
KCl	0	0
NSO ₃	0	0

Most soil arthropods and the two roots afforded enough nutrients to allow Rhizoglyphus to survive and to reproduce (Table 14). The unsuitability of ants and Collembola probably reflects on repellent or even inedible chemical components of their bodies. The persistent, albeit modest, development on Rhizoglyphus cadavers possibly explains how mites survive on filter paper alone, i.e., they feed on their own dead. Earthworms, centipedes and especially isopods afforded much better nutrients, and so did the two roots.

Table 14: Matings and adults of Rhizoglyphus robini obtained after 50 days on macerated invertebrate and root remains

<u>Diet</u>	<u>No. matings</u>	<u>No. adults</u>
Ants	1	0
Collembola	2	0
<u>Rhizoglyphus</u>	19	5
Gamasine mites	32	7
Earthworms	33	26
Centipedes	52	58
Isopoda	394	723
<u>Malva sylvestris</u>	438	446
<u>Silybum marianum</u>	483	811

To conclude, manures, soil invertebrates and roots quite likely enable Rhizoglyphus to survive in the soil and, upon occasion, produce large populations there.

The results of population growth and hypopus production of Rhizoglyphus robini on diets of five soil fungi plus PDA are given in Tables 15 and 16.

In the first trial (Table 15) total population growth was greatest on cultures of Fusarium. Numbers of hypopodes formed was also greater on Fusarium, but the proportion of the population becoming a hypopus was higher in mites fed on Curvularia. Total production on Curvularia however, was only 25% as great as on Fusarium.

Potato dextrose agar provided a good source of nutrition, and appeared to be better than some species of fungi grown on PDA. Sex ratios varied widely among treatments and numbers did not approximate the expected 1:1 ratio. A majority of all populations existed in the juvenile stages rather than as adults. Relatively few adults (ca 4-15%) were present in the cultures and fewer eggs, suggesting an aging population.

In the second test (Table 16), Fusarium again proved to be the best food source based on total numbers of mites produced. However, populations on Botrytis, poor in Test 1, grew large in this test.

As before, the majority of individuals existed as juveniles or eggs regardless of the food source. Few adults were produced but

Table 15. Population growth and hypopus formation of Rhizoglyphus robini on five soil fungi (Trial 1).

Treatment	eggs	Mean number of juveniles	Mean number of hypopodes	Mean no. adults	Mean total reproduction	Mean sex ratio F/M	Mean % juveniles	Mean % hypopodes	Mean % adults
Potato Dextrose Agar	213	4118.8	19.5	205.8	4475.5 b	1.60	91.2 a	0.41 b	8.3
<u>Fusarium oxysporum</u> f. <u>perniciostum</u>	156.5	7201.8	166	189.5	7692.8 a	1.52	93.6 a	2.16 a	4.3
<u>Botrytis gladiolorum</u>	20	51.3	0.3	4.8	59.8 d	1.38	84.8 b	0.51 b	15
<u>Curvularia trifolii</u> f. <u>gladioli</u>	36.8	1698.5	90.8	42.5	1849.5 c	2.36	91.4 a	4.84 a	5
<u>Rhizoctonia solani</u>	34.3	1005	6.8	61	1088.3 cd	4.43	91.4 a	0.86 b	9
<u>Stromatinia gladioli</u>	459.3	1093.3	0.3	133	1282.3 cd	2.75	85.2 b	0.03 b	15

Number of replicates = 4; Initial number of mites = 5 females and 5 males

Excluding hypopodes

Excluding eggs

Based on total reproduction

Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

arcsine transformation was used to compare percentages.

Table 16: Population growth and hypopus formation of Rhizoglyphus robini on five soil fungi (Trial II)

Treatment	eggs	Mean number of juveniles	Mean number of hypopodes	Mean no. adults F	Mean no. adults M	Mean total reproduction	Mean sex ratio F/M	Mean % juveniles	Mean % hypopodes
Potato Dextrose Agar	163.8	120	0	10	2.5	132.5 b	3.8	77.7 a	0 c
<u>Fusarium oxysporum</u> f. <u>perniciiosum</u>	110.5	2044.8	76.5	50.3	54.8	2226.3 a	1.0	91.1 a	3.4 a
<u>Botrytis gladiolorum</u>	53.8	2000	41.3	76.8	52.8	2170.8 a	1.6	88.1 a	2.4 b
<u>Curvularia trifolii</u> f. <u>gladioli</u>	1	2.3	0	4	3	9.3 b	1.6	25.5 c	0 c
<u>Rhizoctonia solani</u>	14.3	74	0	3.3	5.5	82.8 b	1.1	86.4 a	0 c
<u>Stromatinia gladioli</u>	139.5	91.8	0	67.5	34.3	193.5 b	2.6	46.8 b	0 c

Number of replicates = 4; Initial number of mites = 5 females and 5 males

Excluding hypopodes

Excluding eggs

Based on total reproduction

Means followed by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test. An arcsine transformation was used to compare percentages.

in this test the sex ratio approximated 1:1 for mites fed on Fusarium. Hypopus production occurred only on Fusarium and Botrytis, and was greatest on Fusarium. The largest populations in Test 2 were only about 30% as large as the largest in Test 1. The control PDA did not support large populations as in Test 2, and Curvularia, high in the first test produced few mites here.

Table 17 shows the correlation of mite increase and hypopus production with the concentration of nutrients in potato dextrose agar. Population growth on PDA exceeded that on the 1/2 concentration of PDA by nearly 50%. Adults were more than twice as numerous on PDA. In the latter case, no hypopodes were formed. By comparison, in the same test, mites reared on Curvularia grew poorly but formed a relatively large number (38%) of hypopodes. In addition, the cultures of Curvularia contained a free film of water on the agar.

When provided with a choice of daffodil infected with Fusarium oxysporum or non-infected, many mites failed to choose either option but moved about in the dish in an apparently random fashion. More than 87% of the mites making a choice for a diet, in Trial 1, selected the vial containing the infected plant disc (Table 18). Mites congregated on or near the cut disc to feed and lay eggs. In Trial 2 the proportion moving to the infected daffodil was less (62%), but still highly significant. Mites choosing the uninfected daffodil in both trials also fed and laid eggs on the disc. These tissues had begun to deteriorate in some replicates, and thus might have been attractive to mites. Although a few immature stages located the food source, the overwhelming preponderance of mites were adults.

Table 18: Proportion of Rhizoglyphus robini on daffodil infected with Fusarium oxysporum f. perniciosum vs. uninfected daffodil.

Trial I		Trial II	
Mean percent choosing		Mean percent choosing	
Uninfected	Infected	Uninfected	Infected
12.5	87.5	38	62
$z = 5.658$		$z = 2.75$	

Data were analyzed using the Normal Approximation to the Binomial Distribution for One Sample. Preference of mites for the infected daffodil was significant at the 1% level for both Trials.

In a separate trial, incubation of Fusarium inoculated discs for 24 hours followed by a 48 hour choice exposure period, no mites were found on any of the daffodil discs. Eggs were observed on half the infected discs, indicating that adults had visited the food. No eggs were found on the uninfected discs.

Table 17. Population growth and hypopus formation of Rhizoglyphus robini on potato dextrose agar, half-strength PDA, and Curvularia trifolii f. gladioli on PDA (5 females and 2 males at start)

Treatment	Mean number ¹ of		Mean no. adults	Mean total reproduction ³	Mean sex ratio F/M	Mean % juveniles ⁴	Mean % hypopodes ⁴		
	eggs	juveniles ²							
Potato Dextrose Agar	390	796.3	1.0	123.3	43.3	963.8	3.5	82.4	0.1
1/2 Strength PDA	614	434.5	0	52.3	14	500.8	4.3	85.0	0
<u>Curvularia trifolii</u> <u>f. gladioli</u>	12.3	74.5	48.3	12.3	1	136	5.8	52.3	38.2

¹ Based on 4 replicates

² Excluding hypopodes

³ Excluding eggs

⁴ Based on total reproduction

Results of observations on the effect of various foods on transformation of hypopodes are given in Tables 19-22. In the first test of a large quantity of yeast vs. a small quantity, hypopodes without food failed to transform to the tritonymph stage. Addition of yeast in small quantities, four grains or 0.0028 g, resulted in an increase over the non-food check. About 60% (Table 19) molted to resume the life cycle. Addition of greater quantities of yeast (20 grains) resulted in greater transformation (over 80%). In both cases where food was provided greater change was observed during the first few days after exposure.

Table 19: Hypopus transformation with abundant vs. little yeast

Treatment	Mean number ¹ of tritonymphs found on			Mean total hypopodes transformed after 7 days
	Day 2	Day 5	Day 7	
Control (No Food)	0	0	0	0 a
Little Yeast (4 grains)	4.8	3.8	0.8	9.3 b
Abundant Yeast (20 grains)	8.8	3.8	0	12.5 b

¹Initial number of hypopodes per replicate = 15;

number of replicates = 4

Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

Table 20 shows data on hypopus response to different amounts of dry yeast added to each culture. The addition of a food resource, even in minute quantities, resulted in transformation of hypopodes into the tritonymphal stage. Differences among means for food quantity were not significant. Overall, 30% transformed when food was added compared to 2% without food.

Table 20: Hypopus transformation in relation to amount of food

Amount of yeast offered		Mean number of tritonymphs found on day				Mean total number transformed
Grains	Grams	2	4	6	8	
0	0.0	0	0	0.3	0	0.3 a
1	0.0007	1	0.5	0.8	2.3	4.5 b
3	0.0021	1.3	0	0	2.3	3.5 b
9	0.0063	0.3	3.8	0.5	1	5.5 b

¹Initial number of hypopodes per replicate = 15;

number of replicates = 4

Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

In comparison of transformation values on yeast and Curvularia (Table 21): Hypopodes about to transform sought out the Curvularia plug and proceeded to undergo apolysis. This was less obvious when yeast was used but occurred, with tritonymphs and hypopodes crawling on the food. A relatively large number (84.5%) had molted by the fifth day when offered yeast compared to 40.7% of those offered Curvularia. However, each successive observation added to the effect of Curvularia but less so with the yeast. Twenty-five percent of the control had reached the tritonymph stage at the end of 30 days. This suggests that a certain proportion of hypopodes may become tritonymphs regardless of whether or not food is available for them to eat as tritonymphs.

Comparison of hypopus response to three fungi and the PDA check shows no differences after 9 days (Table 22). Initial (3 day) response to Fusarium oxysporum f. perniciosum and Botrytis gladiolorum was significantly greater than response to Curvularia trifolii f. gladioli and PDA in the first trial, but not in the second. Total transformation was similar for the two trials. Addition of PDA, regardless of whether or not a fungus was present, resulted in transformation. The PDA served as a food source for Rhizoglyphus robini.

Table 22: Transformation of Rhizoglyphus robini hypopodes on potato dextrose agar and PDA plus Fusarium oxysporum f. perniciosum, Botrytis gladiolorum, or Curvularia trifolii f. gladioli

Treatment	Mean number ¹ of tritonymphs found on day				Mean total hypopodes transformed after 9 days
	3	5	7	9	
Trial I					
Potato Dextrose Agar	9.5	4	0.3	0.3	14a
<u>Fusarium oxys-</u> <u>porum</u> f. <u>perniciosum</u>	13.5	1	0.3	0	14.8a
<u>Botrytis</u> <u>gladiolorum</u>	15	0	0	0	15a
<u>Curvularia</u> <u>trifolii</u> f. <u>gladioli</u>	8	5	0.5	0.3	13.8a
Trial II					
PDA	11.8	1	0	0	12.8a
<u>Fu. ox.</u>	13.3	0	0.3	0	13.5a
<u>Bo. gl.</u>	10.8	2	0.8	0	13.5a
<u>Cu. tr.</u>	11	2.3	0.8	0	14a

¹Initial number of hypopodes per replicate = 15;

number of replicates = 4

Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

Table 21. Hypopus transformation of Rhizoglyphus robini when offered yeast or Curvularia trifolii f. gladioli.

Treatment	Mean number. of tritonymphs found on day										Mean total number transformed		
	2	5	7	9	12	14	16	19	23	26	28	30	
Control (No Food)	0	1.0	0.8	2.5	0.3	0.5	0.5	0.8	2.3	0.8	0.3	1.0	10.0 a
Yeast	28.8	5.0	0.5	0	0	0.3	0	0.3	0	0	0	0	35.3 b
<u>Curvularia</u>	7.0	9.3	3.8	1.8	1.0	0	0.5	0.8	0.8	0.5	0.3	0.3	26.0 c

Initial number of hypopodes per replicate = 40; number of replicates = 4.

Means followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

3.2.3 The effect of humidity and temperature on hypopodial molting

No hypopodes molted at 87 or 90% r.h., while at least some of those kept at 93% R.H. (with the single exception of 35°) or under more humid conditions apolysed to the tritonymph (Fig. 14). The specific effects of humidity or temperature can be pinpointed by moving along either of the axes, whether percent of molting or average number of days required serve as the indices. When the former was taken as the criterion, 24° (Table 23,a) and 100% r.h. (Table 23,b) yielded the highest values. Upon utilizing the average number of required days (Table 24,a,b) these same conditions brought about the shortest period needed. Moving away from these optimal conditions along either of the axes causes decreasing rates of apolysis, prolonged molting periods, or both.

Better results were consistently obtained under conditions of 100% R.H. than in free water. Very high temperature (35°) usually hindered apolysis, this effect being noticeable at all humidities (except possibly at 100% R.H.). Highest molting rates of hypopodes of another acarid, Caloglyphus mycophagus (Megnin), on the other hand, were obtained at 35° (Kuo and Nesbitt, 1970).

Table 23: Molting of hypopodes of Rhizoglyphus robini (Expressed as average percentages) as affected by temperatures (a) and by relative humidities (b) (FW: free water).

<u>a</u>		<u>b</u>	
<u>Temperature</u>	<u>Average percent of molt</u>	<u>Relative humidity</u>	<u>Average percent of molt</u>
16°	88.3	93	51.2
24°	93.2	96	68.9
27°	67.0	100	97.5
35°	51.6	FW	82.4

Table 24: Periods (in days) required by the hypopodes of Rhizoglyphus robini for molting, as affected by temperatures (a) and by relative humidities (b) (FW: free water).

<u>a</u>		<u>b</u>	
<u>Temperature</u>	<u>Days</u>	<u>Relative humidity</u>	<u>Days</u>
16°	20.8	93	13.6
24°	4.8	96	14.6
27°	10.3	100	7.7
35°	10.9	FW	11.6

Sex ratio of reared tritonymphs was 1.3 : 1.0, males to females. This differs from the 1:1 ratio obtained in the life history studies and could have been due to the smallness of the batch. It might also reflect on some sex-related differences in development. Whatever the cause, the results establish that both sexes of R. robini may have a deutonymphal instar in their ontogeny.

Fig. 14. Molting percentages of hypopodes of *Rhizoglyphus robini* and the average number of days required for this process as affected by various temperature and humidity conditions. Unmarked numbers and columns signify percentages, underlined numbers and striped columns denote days. FW = free water.

Ferro and Chapman (1979), who studied the hatch rate of spider mite eggs, recommended that saturation deficit (SD) or some other index of absolute humidity be favored over r.h. percentages when examining the effects of temperature and humidity on an organism. It thus became of interest to test the applicability of their argument to the present study. SD values were calculated with the equation

$$SD = E_s (1 - \text{----})$$

where E_s denotes mmHg at any given temperature.

Table 25: Molting or non-molting of hypopodes of R. robini as affected by combinations of saturation deficit and temperatures (T). Relative humidity (r.h.) values are added for comparison. (FW: free water).

<u>Saturation deficit(%)</u>	<u>T=°C</u>	<u>r.h. (%)</u>	<u>Molt</u>
0.0	16°	FW	+
0.0	24°	FW	+
0.0	27°	FW	+
0.0	35°	FW	+
0.0	16°	100	+
0.0	24°	100	+
0.0	27°	100	+
0.0	35°	100	+
0.5	16°	96	+
0.9	24°	96	+
0.9	16°	93	+
1.1	27°	96	+
1.4	16°	90	-
1.6	24°	93	+
1.7	35°	96	+
1.8	16°	87	-
1.9	27°	93	+
2.2	24°	90	-
2.7	27°	90	-
2.9	35°	93	-
2.9	24°	87	-
3.5	27°	87	-
4.2	35°	90	-
5.5	35°	87	-

No molting took place when SD exceeded 1.9 (Table 25), but hypopodes also failed to transform at SD of 1.4 and 1.8. Both exceptions occurred when mites were held at 16°C. Upon using r.h. as the criterion, Table 25 shows that molting took place regularly at, or above, 93% except at 35°C. These exceptions may thus be attributed to specific effects of extreme temperatures. To a certain extent they cancel each other. However, the use of SD values alone would have masked the adverse effects of free water on molting.

In tests in Virginia of hypopodes kept six months at 4°C, 57% transformed. For those kept at 26°C, 37% transformed during the first month, and 8% during the second, making 45% total. It was concluded that six months at low (4°C) temperature did not appear to reduce the ability of the hypopus to transform into the nymphal stage. In fact, there was a higher percentage of tritonymphs in this test than in the test held at 26°C.

3.2.4 Effects of three diets on body and setal size

The various diets had a marked and significant effect on all parameters measured (Table 26), while variation remained uniformly low (except in regard to setae sc i). The effect, however, was uneven: lengths of body, leg I and even setae sc e decreased by about the same ratio from peanut seeds to peanut extract and from there to filter paper (Table 27), but the ratio for sc i was different. This deviation is even more marked upon comparing the length ratios between mites reared on peanut seeds and filter paper (1.7-1.9 for the first three parameters, 2.9 for setae sc i). Upon comparing these measurements with each other as affected by the various diets (Table 28), it becomes evident that setae sc i were relatively shorter on mites with the smallest bodies, longest on the largest (almost by a factor of two). Allometric variation was also obtained in relation to setae sc i and leg I as well as setae sc e, the pair closest to sc i (Fig. 15-1). Length ratios of other parameters were not affected by the various diets.

Table 26: The effect of three diets on length (in μm) of body, leg I, setae sc e and setae sc i of males of *Rhizoglyphus robini*. CV denotes coefficient of variation ($\text{CV} = \text{S.D.}/\text{X}$), expressed in percentages. Different letters following means signify differences, $P < 0.01$, using Duncan's multiple range test.

Diets	Body		Leg I		Setae <u>sc e</u>		Setae <u>sc i</u>	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Peanuts	643.2a	8.7	196.6a	10.9	146.3a	10.6	7.2a	14.3
Peanut extract	468.7b	8.6	147.9b	11.1	106.1b	13.8	4.1b	26.2
Filter paper	377.1c	7.7	117.5c	10.1	75.5c	11.9	2.5c	19.2

Fig. 15: Micrographs of males of *Rhizoglyphus robini*. 1. Dorsum of propodosoma, showing setae *sc e* and *sc i* and, to define propodosoma, setae *vi*. 2. Venter of hysterosoma, showing closed genital valves (GV), anus (A) and anal suckers (AS). 3. Closed genital valves, enlarged. 4. Venter of hysterosoma; genital valves open and penis (P) partly extruded. Anus with excretion. White bars in 15-1 and 15-4 denote 100 μm, in 15-2 and 15-3, 10 μm.

Table 27: Ratios between lengths of body and some appendages measured on males of Rhizoglyphus robini fed various diets.

<u>Diets</u>	<u>Peanuts/ peanut extract</u>	<u>Peanut extract/ filter paper</u>	<u>Peanuts/ filter paper</u>
<u>Ratios</u>			
Body/body	1.4	1.2	1.7
Leg I/Leg I	1.3	1.3	1.7
<u>sc e/sc e</u>	1.4	1.4	1.9
<u>sc i/sc i</u>	1.8	1.6	2.9

Table 28: Ratios between length of body and different appendages of males of Rhizoglyphus robini from the same diets

<u>Diets</u>	<u>Peanuts</u>	<u>Peanut extract</u>	<u>Filter paper</u>
<u>Ratios</u>			
Body/leg I	3.3	3.2	3.2
Body/sc e	4.4	4.4	5.0
Body/sc i	89.3	114.3	150.8
Leg I/sc e	1.3	1.4	1.6
Leg I/sc i	27.3	36.1	47.0
sc e/sc i	20.3	25.9	30.2

Size variation in males of R. robini was noted by Hughes (1976), who stated that they may measure 450-720 μm . Size is known to be a major cause of allometric growth, which brings about allometric variation (Mayr, 1969). Czajkowska (1972) offered many medicinal plants to R. echinopus and reported that there were almost three-fold differences in mite size between those reared on unsuitable herbs and those on the control diet. It is thus reasonable to postulate the occurrence of similar variations in the length of setae sc i in R. robini also. Such variation should be considered while using this character in the definition of Rhizoglyphus spp.

SEM microscopy showed the male genital valves of R. robini. The valves are elongated, with lower conical supporting struts and clearly bilobed (Figs. 15-2,3); Hughes (1976), in her Fig. 136, also showed the genital valves to consist of two parts. The valves open posteriorly to extrude the penis (Fig. 15-4). At mating the male extends its penis backward, directly into the female's bursa copulatrix (they copulate end-to-end, with the male's opisthosoma being placed above that of the female, Fig. 6). The penis shown in Fig. 15-4 was probably either in the process of being extended or of being withdrawn.

3.2.5 Miscellaneous observations

3.2.5.1 Survival under water

Soil mites may at times become totally immersed in water, due to heavy rains, flooding or irrigation, and it became of interest to gauge the ability of Rhizoglyphus to survive under such stress. This could also bear on the mites' ability to disperse with water movement in or above soil.

Twenty-five mite couples were put in a glass containing 40 ml of distilled water (to reduce any possible electrolyte effect) which was placed at 22°C. Some mites immediately sank while others remained at the water's surface. Ten couples were removed after four weeks and were alive; the others were taken out a week later. Five mites were dead, the rest almost evenly divided between males and females. Both groups were then subdivided. Some males were placed with virgin females, the others with ovipositing ones. Some females were isolated without males (to see whether, insofar as they had already mated, they would still oviposit), the others with young males. All were kept in padded petri dishes at 22°C and offered garlic cloves.

Some mortality occurred in all groups. Males placed with virgin or older females soon mated and females in both groups soon oviposited dozens of viable eggs. Five of these males lived for four weeks after their water ordeal. Two of the isolated females produced, respectively, 23 and 16 viable eggs; the others died or did not oviposit. Females placed with young males readily mated and deposited many viable eggs.

It is concluded that a continuous five week long immersion in water would not seriously affect the survival of Rhizoglyphus in the soil, and that flooding cannot be considered as a means of control.

3.2.5.2 Survival in cold water

In continuation of the above observation, 100 couples of Rhizoglyphus were placed in a glass as above, at 6-8°C, in a refrigerator. Samples were removed after two, four and six weeks, checked for living and dead mites, and the former placed on garlic cloves. Survival rates were 65% after two weeks, 75% after four weeks and 71% at the end of six weeks. Many surviving females from all groups subsequently deposited viable eggs. The winter temperatures prevailing in Israel would thus not have an important effect on Rhizoglyphus survival.

3.3 An enzymatic method for separating mites from plant tissue

Appraising the numbers of Rhizoglyphus infesting onion or garlic by direct counts is tedious, difficult (in part due to the tear-inducing smell), time consuming and error prone. Errors result from the observer's inability, while counting large populations, to distinguish between counted and uncounted individuals. A simpler, quicker and more precise method was therefore sought. Initial efforts included macerating plant tissues by mechanical means and trying to separate the mites from the resultant macerate, boiling plant tissues in acids or bases, and various combinations of these two groups of methods.

An enzymatic preparation was finally tried, made from two enzymes: a pectinase and a cellulase. The pectinase was poly- α -1,4-galacturonide glycano-hydrolase, and the cellulase, 1,4-(1,3,1,4)- β -D-Glucan 4 glucano-hydrolase. Both were supplied by Sigma, and were used at 2.5% solutions in 30 ml buffer acetate (pH = 4.4). This preparation was used to dissolve the infested plant material. The mixture of dissolved plant material, diverse debris and mites will be termed the raw product. To separate mites from this raw product benzene and xylene were used. Both do not mix with, and are lighter than, water and have an affinity for the mites' integument. The freezing point of benzene is above that of water, therefore it floats above the raw product and may then be removed, evaporated in a hood and the mites counted. However, it is known to be carcinogenic. Xylene, on the other hand, has a lower freezing point than water. The latter, frozen, is then removed, the xylene is placed under a hood and evaporated, and the mites counted.

In practice plant material was cut twice, placed in Ehrlenmeyer flasks (100 ml) together with the enzymatic preparation and shaken in warm water (29°C) for five days. Good dissolution of the onions or garlic was obtained by that time. This raw product was then treated as described.

Some experiments were run to assay the efficacy of this method. Two hundred adult mites were placed in the enzymatic preparation and extracted with benzene. A total of 185 (92.5%) were recovered, the rest having probably been lost on the inner sides of the flasks or during manipulation. Pieces of onion and garlic (separately) were next placed in the preparation, along with 20 mites in each of five flasks. Recovery came to 92%. Sand, which usually occurs in field-collected samples, was added in the next set of experiments. These were run in various combinations of treatments: (a) sand with 80 mites, extracted by benzene; (b) sand with 80 mites and garlic, extracted by benzene; and (c) sand with 185 mites and garlic, extracted by xylene. The overall recovery rate (Table 29) was over 90%.

A variable number of mites was placed by another person in each of eight flasks in the next experiment, so that the observer did not know the size of the introduced batch. Mite-free soil and bits of garlic were also put in the flasks. Recovery was between 70-100% and the average came to 90.8%. In this as well as in the final experiment mites were recovered with xylene.

Table 29. Recovery of Rhizoglyphus robini from the enzymatic preparation with benzene and (a) sand alone; (b) and garlic, and (c) with xylene from sand and garlic.

Experiment	Replicate	Mites				Experiment	Replicate	Mites			
		Introduced	Recovered	%				Introduced	Recovered	%	
a	1	20	20	100	c		1	15	14	93.3	
	2	20	20	100			2	10	7	70.0	
	3	20	16	80			3	40	39	97.5	
	4	20	19	95			4	45	44	97.8	
	Totals and average	80	75	93.5			5	20	20	100	
b	1	20	19	19	Totals and average		6	10	9	90.0	
	2	20	20	100			7	25	18	72.0	
	3	20	15	75			8	20	17	85.0	
	4	20	20	100					168	90.8	
	Totals and average	80	74	92.5					185		

As a last step the method was tried with field-collected material, i.e., carrying an unknown number of mites. A sample of 18 heavily infested garlic plants was examined by visual means (with a stereoscopic microscope) to establish the number of mites found by this method. The plants were then treated by the enzymatic method and the numbers of mites were compared (Table 30). Overall, the enzymatic method extracted 1252 (47.6%) more mites than were detected by visual examination using the microscope but the values obtained by the latter means were higher in five (28%) cases. An estimate of the time required was 17 minutes/plant for the microscopic method, 23 minutes/plant for the enzymatic one. However, the latter is far less tedious; following extraction the flasks may be kept and mites counted as convenient. And finally, yield of the enzymatic method was considerably higher, thereby affording a far better estimate of the mites' actual populations in and on infested plants.

Table 30: Numbers of Rhizoglyphus robini counted on and in, and extracted by the enzymatic method from, 18 infested garlic plants.

<u>Plant (sample)</u>	<u>Microscopic method</u>	<u>Enzymatic method</u>	<u>Difference</u>
1	390	291	-99
2	0	3	3
3	82	108	26
4	435	1034	599
5	53	2	-51
6	8	42	34
7	120	414	294
8	35	159	124
9	54	116	62
10	3	41	38
11	173	150	-23
12	252	283	31
13	590	487	-103
14	25	121	96
15	39	54	15
16	59	211	152
17	255	330	75
18	55	34	-21
Total	2628	3880	1252

3.4 Discussion

The threshold of development of R. echinopus was calculated by Kuznetsov and Tkatchuk (1972) to be 9.7°C , and an average of 180 day-degrees were therefore required to complete a generation. Our computed threshold (11.8°C) was well above the reported one, but the sum of day-degrees (184.8) for R. robini was quite similar. Most authors who studied Rhizoglyphus (Garman, 1937; Kuznetsov and Tkatchuk, 1972; Sekiya, 1948; Woodring, 1969) noted that the egg stage may last for a third or even more of the entire immature period. Our results (Table 11) are in general agreement, except that larval and nymphal development

became prolonged under adverse conditions, which did not affect the relative duration of the egg period.

Food quality had a marked effect on the development and fecundity of R. robini (Tables 11 and 12, Fig. 10). It also strongly affected the frequency of mating (Fig. 8, Tables 13, 14), as mites offered richer foods mated more frequently than those on poorer diets. The influence of food quality on changes in this mite's populations is thus made up of several components. These include survival of immatures, development time, mating frequency and thereby the length of time to first oviposition, and number of progeny produced (which may be affected by size of individuals which had grown on that diet, in addition to the latter's specific nutritive value). The compounded effect of these factors (and possibly others, such as male potency, not investigated in this context) probably causes great fluctuations in mite numbers under natural conditions. Another confusing factor could be the influence of poor nutrition. Results obtained with R. robini reared on filter paper suggest that were such starved mites to be offered richer foods, they could recover and produce viable progeny. However, the low immature survival rates as well as quite smaller bodies indicate that their populations would require at least another generation to recover.

Such considerations point to the need for precise definitions of diets on which polyphagous mites (such as Rhizoglyphus) are reared in life history studies. The rather variable development, longevity, fecundity and survival results which Czajkowska (1972) obtained upon offering 52 species of medicinal herbs to R. echinopus lend support to our contention. All this helps to explain the different results reported in some former life history studies (apart from taxonomic problems, as noted above). Life table parameters calculated from such variable data would also be rather variable.

The fecundity values of R. robini on garlic approximate those reported by Sekiya (1948), who reared R. echinopus on Allium bakeri (460 eggs/female at 24°C, 436 eggs/female at 27°C). They were, however, considerably higher than the value (109 eggs/female on gladiolus corms at 18-25°C) reported by Kuznetzov and Tkatchuck (1972, Table 6) for the same species. Woodring (1969), who kept R. echinopus on chopped fresh yellow mealworms (larvae of the beetle Tenebrio molitor L.), a diet presumably rich in proteins, obtained an average of 285 eggs/female, the maximum being 630. All these fecundity rates are well below the values reported herein for mites reared on peanuts, and even these values might have been higher had more than one male been placed with each female. Woodring (1969) noted that replacement of one male by another enhanced the fecundity of some (but not all) females.

The apparent sterility induced in mites reared at 35°C bears on results obtained while demonstrating control of R. robini in the soil by solar heating. Mites in these experiments were completely eliminated down to depths of 20 and 30 cm, where maximum temperatures reached 38°C. The data presented above indicate that mites which might have survived solar heating would probably have become infertile.

The prolonged periods of oviposition (Figs. 11 and 12) extended well over at least two, and possibly three, mite generations, thereby causing their overlapping. A single mated female may therefore raise a considerable population in its own life time. This could explain the large numbers of R. robini found on infested bulbs in the fields.

Kuznetsov and Tkatchuk (1972) reported that males lived somewhat longer than females (at 18-25°C), 38.2 days on the average as compared to 31.7 days (a difference of about 20%). In the present study males lived almost twice as long as females. As R. robini does not reproduce by parthenogenesis, prolonged male longevity increases the chances that virgin females, isolated in the soil, would meet their mates.

The r_m values obtained by the simplified Wyatt and White method (Table 12) for mites which had developed on peanuts and garlic at 27°C were quite similar to those calculated by conventional methods. At 16°C, and with the mean generation values, the results were too different to be acceptable. The deviation is probably due to the oviposition pattern of R. robini, which does not deposit 95% of its eggs during the 2d period. And yet, the good fit obtained in regard to r_m values suggests that this simplified method may have wider applicability than visualized by Wyatt and White (1977).

The feeding experiments afford some insight into how various organic materials - manure, plant roots, invertebrate remains - influence the populations of Rhizoglyphus in the field. Results obtained with the manures were especially significant in this respect, and possibly explain mite outbreaks following manure application. Animal as well as plant substrates allowed Rhizoglyphus to raise large populations; this supplements Barabanova's (1976) observations on the rapid and versatile responses of these mites' digestive enzymes.

The occurrence of molds in one of the NPK experiments and their beneficial effect on Rhizoglyphus survival probably reflects similar situations under natural conditions. Feeding on molds which develop on fertilizers (or any of the other substrates assayed in Tables 13 and 14) only moves the mites one step along the food chain. From being a primary consumer feeding on a producer (i.e., roots), the mite becomes a secondary consumer feeding on primary consumers (molds). This again attests to the great versatility in the feeding habits of Rhizoglyphus.

Hypopodes molted best under saturation conditions, obtained with distilled water (Fig. 14). Various authors have reported on diverse environmental cues, like fresh foods, salts, precooling or heating, which induce apolysis in deutonymphs of different Astigmata, usually under high humidities. Thus while all hypopodes require high humidity (or low SD) to molt, the precise prerequisites differ among species.

Hypopodes were observed to molt when isolated with fresh sources of food. Fusarium, yeast, and other foods elicited similar molting response.

4. Cultural and Chemical management

Rhizoglyphus mites may remain in the soil throughout their lives, their presence being manifested only by the damage they cause. Management practices therefore depend, first and foremost, on being aware of mite presence. Once it is known, two options are available. Mites may be controlled prior to planting (by soil disinfection), or when the plants are already standing in the field, by various pesticides.

4.1 Disinfection: pre-planting control

4.1.1 Cultural methods and results

Solar heating of the soil by means of covering it with transparent polyethylene sheets has been developed in Israel to control soil-borne pathogens, certain nematodes and weeds (Katan, 1981). The wide applicability of this method and its nonchemical nature suggested that it might be feasible against Rhizoglyphus. Solar heating must be implemented during mid-summer, i.e., long before onion or garlic is sown or planted.

Infested peanut seeds were placed in small perforated nylon bags. Each seed was accompanied by two noninfested seeds, intended to provide sufficient food for the mites. The seeds were then buried at the desired depths in a shadeless, cultivated plot in the coastal plain. The treated plots were covered by transparent polyethylene sheets, 40 μ thick, while the nontreated control plots, in which the same number of infested seeds were buried at the same depths, remained uncovered. Experiment A (10 and 20 cm depths) was replicated three times and the seeds were examined after being buried for 10, 20 and 30 days. Experiment B (10 and 30 cm depths) was replicated four times, and seeds were also examined after 10, 20 and 30 days. The experiments were conducted during July and August. Post-treatment monitoring (by trapping) in those plots continued for seven months longer.

The vast numbers of mites which developed in some of the controls precluded counting, and a ranking method was used instead. Mite numbers were estimated and ranked accordingly, on a logarithmic scale, of 0 = no mites, 1 = very few mites, 2 = ten to a few dozen, 3 = hundreds, and 4 = thousands of mites. The ranks were then multiplied by the appropriate factor: 1 = 1, 2 = 10, 3 = 100 and 4 = 1000. Values thus obtained from the various replicates of each treatment were added up and divided by the number of replicates. For instance, in Experiment A after 20 days, at 10 cm, the ranks for the three replicates in the controls were 3, 3, and 4. Multiplying them by their appropriate factors ($1 \times 100 + 1 \times 100 + 1 \times 1000$), one obtains 1200, which, when divided by the number of replicates, 3, provides the result for that date: 400.

Mites were completely exterminated in the solar-heated plots at the 10 and 20 cm depths (Experiment A in Table 31). At 30 cm (Experiment B) there was some survival after 20 days, probably because of the lower temperatures at this depth. By day 30, however, none was alive.

Table 31: Estimated survival of Rhizoglyphus robini (appraised by ranking) following solar heating of the soil

Soil treatment	Experiment A ^a					
	Days after polyethylene mulching					
	10	20	20	30		
	Depth (cm)					
Untreated	10	20	10	20	10	20
Solar heated	37	700	400	700	370	70
	0	0	0	0	0	0
	Experiment B ^b					
	Days after polyethylene mulching					
	10	20	20	30		
	Depth (cm)					
Untreated	10	30	10	30	10	30
Solar heated	32	77	100	1000	325	750
	0	77	0	77	0	0

^aIn solar-heated soils the maximum temperatures at depths of 10 and 20 cm were 45° and 38°C, and the minimum temperatures were 30° and 31°C respectively. In the untreated soils the maximum temperatures were 8-12°C lower.

^bIn solar-heated soils the maximum temperatures at depths of 10 and 30 cm were 48° and 38°C, and the minimum temperatures were 31° and 32°C respectively. In the unheated soils the maximum temperatures were 7-12°C lower.

During the following months populations in the treated plots were only about one tenth of those in the untreated controls. By the next spring (seven months post-treatment) mite numbers in the former plots were about half those in the controls.

These data provide the first demonstration of the possibility of controlling a soil pest by solar heating of the soil.

Another possible cultural method of limiting Rhizoglyphus damage could be the use of tolerant garlic varieties, as indicated in section 2.2.3.

4.1.2 Chemical methods and results

Chemical disinfectants are faster acting than solar heating, but a period of time must be allowed for toxicant decomposition and soil aeration prior to planting. Two materials were tried: DBCD (Nemagon, 1,2-dibromo-3-chloropropane) and Vapam (metham-sodium).

DBCD: this material was injected into the soil under pressure at the rate of 100-120 liter/ha, during August, in a plot where Rhizoglyphus was found and which was being prepared for a flower crop. Mite activity was monitored from November till April. Only one trap, in late March, had a "positive". This chemical thus appeared to be very effective against mites in the soil.

Vapam had been tried against Rhizoglyphus by Jefferson et al., (1956), with some success. Here it was tried in a large commercial field formerly planted to onions which had become heavily infested. The toxicant was applied in 600 m³ irrigation water through the overhead sprinkling system, dosages of 600 and 1000 liter/hectare being tried; the control plot was concurrently sprinkled without Vapam. Infested leguminous seeds were placed in the soil (see 4.1.1) one day before treatment, at depths of five and ten cm, and examined one day later.

Vapam was applied in July. Potatoes were later planted in the entire field, and Rhizoglyphus monitoring was undertaken until late December.

All mites exposed to the two Vapam concentrations were dead by the next day, while those in the control remained alive and thriving. But monitoring in the autumn (Table 32) indicated that the treated plots had become recontaminated within three months; the effect of Vapam thus appears to be rather transient.

Table 32: Trapping Rhizoglyphus in soil treated with 1000 liter/hectare (a) or 600 liter/hectare (b) Vapam, and in the control plot (c) (symbols as in Table 1).

	<u>a</u>		<u>b</u>		<u>c</u>	
	<u>TE</u>	<u>%P</u>	<u>TE</u>	<u>%P</u>	<u>TE</u>	<u>%P</u>
October	14	14	12	42	11	45
November	22	18	22	27	20	20
December	6	16	6	16	6	0

4.2 Pesticides: post-planting control

4.2.1 Granulated pesticides

Four systemic compounds, all granulated at 10% a. i., were first tested in the greenhouse and then in the field. The pesticides were Aldicarb (Temik), Dimethoate (Rogor), Oxamyl (Vydate) and Phorate (Thimet).

In the greenhouses the pesticides were mixed with soil at a rate equivalent to 50 kg/hectare of the upper 10 cm layer and placed in wooden boxes. To simulate natural growing conditions garlic was planted in the center of each box, in a row. Infested leguminous seeds in nylon bags (as in 4.1.1) were introduced into the treated soil to a depth of three cm, about two cm away from the garlic plants. These introductions were repeated four times, 11, 21, 47 and 54 days post-treatment and held in the soil for 7-9 days, after which they were recovered and examined. The same pesticides were then tried in early spring, in an infested onion field. Compounds were introduced into the soil as side dressing between two rows, applied by a manual sowing planter, at the rate of 2 kg/1000 m². Each pesticide was tried in a 120 m long bed holding five onion rows, and was monitored by ten traps (30-40/month). The number of plants pre- and post-treatment was counted.

Total, rapid mite mortality was obtained only with Aldicarb and Phorate (Table 33), the latter compound being effective also after four weeks. All pesticides were ineffective eight weeks post treatment.

Table 33: Survival of Rhizoglyphus exposed to granular formulations of four pesticides in the soil (A and B denote, respectively, the number of days after treatment when the mites were introduced, and when they were examined; - : no mites; + : single mites present; ++ : dozens of mites present; +++ : hundreds of mites present).

A	11	21	47	54
B	20	29	54	67
Aldicarb	0	+	+	+++
Dimethoate	++	+++	(not checked)	+++
Oxamyl	+	++	+	+++
Phorate	0	0	+	++

Results of the field experiment (Table 34) were rather inconclusive and uneven, the somewhat better performance of Oxamyl being in disagreement with the data presented in Table 33. The uniform May reduction was most likely caused by seasonal factors.

Table 34: Percentage of "positive" Rhizoglyphus traps in an onion field treated with four granular pesticides (a) and number of plant/m at pesticide application time and three months later (b).

	a				
	Control	Aldicarb	Dimethoate	Oxamyl	Phorate
February	40.0	36.6	40.0	23.3	26.2
March	43.3	43.3	33.3	43.3	30.0
April	62.5	57.5	67.5	42.5	40.0
May	25.0	17.5	12.5	12.5	22.5
	b				
February	9.7	10.7	10.2	10.1	11.5
May	8.0	8.6	9.3	9.6	9.2

4.2.2 Pesticides in liquid form

Chemical control methods of Rhizoglyphus mites include dipping of infested plant parts like bulbs and corms in formulations of soluble pesticides (Schread, 1969; Tanaka et al, 1962 and Wilkin et al, 1976). In an infested field pesticides can also be applied as soil drenches at various stages of growth of the plants (Schread, 1969).

The effect of a wide range of pesticides on Rhizoglyphus was tested in the laboratory. The basic slide-dip method of Dittrich (1962) was employed, with a slight modification. The dorsum of Rhizoglyphus does not adhere to two-sided sticky scotch tape. Mites were consequently placed onto masking tape which was then glued onto the scotch tape.

Only young females were used, 10/slide and 4 slides or replicates per pesticide concentration.

Slides with mites were dipped into the toxicant for five seconds (control mites in tap water) and then held for 24 hours in saturation humidity. Total lack of mite movement, even when probed by brush, was the criterion for mortality. Tests were rerun in cases of excessive control mortality. All data were corrected by Abbott's formula (Abbott, 1925).

All pesticides were tried in four concentrations. Pesticides tested belong to different chemical groups. 1 & 2: Organo-chlorine and organotin compounds, conventional and well established acaricides. 3. Organophosphate insecticides known also for acaricidal activity or nematocidal activity. 4. Carbamate compounds, known for nematocidal activity or recommended for soil drench treatment. 5. Derivatives of various compounds known as acaricides or with an indication of acaricidal activity. 6. Synthetic pyrethroids known for their broad spectrum activity.

The compounds tested are listed in Table 35 according to these groups giving the official common name, some of the trade names, empirical formula, percent active ingredient, formulation and manufacturer according to Pesticide Manual (Worthing, 1980) and Agricultural Chemicals (Thomson, 1982-83).

Table 35. Compounds tested for effect on R. robini
(EC-emulsifiable concentrate, SL-soluble
liquid, WP-wettable powder; FM-Formulation).

Common (Trade) Name	Empirical formula	% Act. Ing.	FM	Manufacturer
<u>1. Organochlorine compounds</u>				
Dicofol (Kelthane, Acarin)	$C_{14}H_9Cl_5O$	18%	EC	M.C.W. Israel
	and	18.5%	WP	
Chlorobenzilate (Benzilan)	$C_{16}H_{14}Cl_2O_3$	25%	EC	M.C.W. Israel
Bromopropylate (Neoron, Acarol)	$C_{17}H_{16}Br_2O_3$	50%	EC	CIBA-Geigy Co. Switzerland
Tetradifon (Tedion)	$C_{12}H_6Cl_4O_4O_2S$	8%	EC	Duphar, Holland
Dienchlor (Pentac)	$C_{10}Cl_{10}$	50%	WP	Hooker Chemical Co. U.S.A.
<u>2. Organotin compounds</u>				
Cyhexatin (Plictran)	$C_{18}H_{34}OSn$	50%	SL	Dow Chemical Co. U.S.A.
	and	25%	WP	
Cyhexatin + Tetradifon (Durbart)		15% + 8%		Dow Chemical Co. U.S.A.
Azocyclotin (Peropal)	$C_{20}H_{35}N_3Sn$	25%	WP	Bayer AG Germany
<u>3. Organo-phosphate compounds</u>				
Azinophosmethyl (Gusathion, Guthion, Cotnion)	$C_{10}H_{12}N_3O_3$	20%	EC	M.C.W. Israel
Chlorpyrifos (Dursban, Pyrinex)	$C_9H_{11}Cl_3NO_3PS$	25%	EC	M.C.W. Israel
Diazinon (Basudin)	$C_{12}H_{21}N_2O_3PS$	25%	EC	M.C.W. Israel
Dimethoate (Rogor)	$C_5H_{12}NO_3PS_2$	40%	EC	Montedison, Italy
Ethion (Itopaz)	$C_9H_{22}O_4P_2S_4$	50%	EC	Pazchem, Israel
Fenamiphos (Nemacur)	$C_{13}H_{22}NO_3PS$	40%	EC	Bayer, AG Germany
Fenthion (Lebacycid)	$C_{10}H_{15}O_3PS_2$	56.6%	EC	Bayer, AG Germany
Methamidophos (Tamaron, Monitor)	$C_2H_8NO_2PS$	60%	EC	Bayer, AG Germany
Omethoate (Folimat)	$C_5H_{12}NO_4PS$	58.8%	EC	Bayer, AG Germany
Triazophos (Hostathion)	$C_{12}H_{16}N_2O_3PS$	40%	EC	Hoechst, German

4. Carbamate compounds

Bendiocarb (Ficam)	$C_{11}H_{13}NO_4$	80%	WP	Fisons UK
Butocarboxim (Darwin 755)	$C_7H_{14}N_2O_2S$	50%	SL	Wecker Chemie Germany
Ethiocarb (Croneton)	$C_{11}H_{15}NO_2S$	50%	EC	Bayer, AG Germany
Methomyl (Lannate)	$C_5H_{10}N_2O_2S$	50%	EC	DuPont US
Oxamyl (Vydate)	$C_7H_{13}N_3O_3S$	25%	SL	DuPont US
Promecarb (Carbamult)	$C_{12}H_{17}NO_2$	25%	EC	Schering AG Germany
Dioxacarb (Elocron)	$C_{11}H_{13}NO_4$	40%	EC	CIBA-Geigy Switzerland

5. Derivatives of various compounds

Dinobuton (Acres)	$C_{14}H_{18}N_2O_7$	30%	EC	Keno-Gard Sweden
Propargite (Omite)	$C_{19}H_{26}O_4S$	30%	WP	Uniroyal U.S.A.
Amitraz (Mitac, Mitex)	$C_{19}H_{23}N_3$	10%	EC	Boots Co Ltd
Fluthiamin (Cropotex)	$C_{17}H_{10}F_6N_4S$	50%	WP	Bayer, AG Germany
MK 936 Avermectin B ₁				
80% a.	$C_{48}H_{72}O_{14}$	0.36	SL	Merck Sharp & Dhorne U.S.A.
20% b.	$C_{47}H_{70}O_{14}$			
Apollo (NC21314)	$C_{14}H_8Cl_2N_4$	50%	WP	Fisons Ltd U.K.

6. Synthetic pyrethroids

Cypermethrin (Cymbush)	$C_{22}H_{15}Cl_2NO_3$	10%	EC	I C I UK
Fenvalerate (Sumicidin, Aomatrine)	$C_{25}H_{22}ClNO_3$	20%	SL	Sumito Chemical Co. Japan
Fenpropathrin (Meothrin, Smash)	$C_{22}H_{23}NO_3$	20%	SL	Sumito Chemical Co. Japan
Flucythrinae (Cybolt, AC 22705)	$C_{26}H_{23}F_2NO_4$	30%	SL	American Cyanamid
Permethrin (Ambush)	$C_{21}H_{20}Cl_2O_3$	25%	SL	I C I UK

The mortality rates obtained in the tests are presented in Figures 16-21, according to the chemical groups and active ingredient concentrations.

1. The organochlorine compounds tested are specific well established acaricides. All were effective in the higher concentrations as seen in Fig. 16. Tetradifon (Tedion), which is not known to affect adults, was quite effective, even at low concentrations. The two Dicofol formulations were similar in action.

2. The organotin compounds tested were specific acaricides, and they all gave high mortality at the higher concentrations, as shown in Fig. 17. The Cyhexatin (Plictran), as wettable powder, much exceeded the soluble liquid at 0.05% a.i. The addition of Tetradifon (Tedion) to Cyhexatin (Durbart) increased mortality, especially at low concentrations.

3. Among the organophosphate compounds tested only Omethoate (Folimat) is considered an acaricide-insecticide, Fenamiphos (Nemacur) is a nematocide. The rest are known as broad spectrum insecticides with acaricidal action. All organophosphates tested gave high kill at the concentrations used, as seen in Fig. 18. Even the lowest concentrations exceeded 50% kill.

4. Of the carbamates tested, Oxamyl (Vydate) is described as a nematocide controlling mites, Butocarboxim (Darwin) as suppressing mites, the rest are described as affecting soil pests and used as soil drenches. However, all carbamate compounds were effective at concentrations used as shown in Fig. 19.

5. Among derivatives of various compounds Dinobuton (Acrex) and Propargite (Omite) are known acaricides, and Amitraz (Mitex) is supposed to affect all stages of mites. However, at the concentrations tested they did not yield sufficient mortality rates, except Apollo, and should be tried at higher concentrations (Fig. 20).

6. Of the pyrethroids, only Fenpropathrin (Meothrin, Smash) is mentioned for acaricidal activity (Thomson 1982-83). The rest are known for their highly effective broad spectrum activity and are recommended for use at low concentrations. However, only Fenpropathrin (Smash) and Permethrin (Ambush) reached 50% mortality at the lower concentrations, while the higher concentrations were disappointing (Fig. 21).

Most of the pesticides tested afforded adequate kill of mites by direct contact and can be recommended for dipping plant parts, like bulbs and corms, or for soil drenching. However, the mites present on bulbs or corms may not always be exposed to direct contact with pesticides, as they may be covered by scales or leaves. This may account for unsuccessful results in dipping bulbs or corms (Englehard, 1967).

Fig. 16. Percent Mortality of R. robini mites after dipping in organochlorine compounds (inside each column % a.i.).

Fig. 17. Percent mortality of R. robini mites after dipping in organotin compounds (inside each column % a.i.).

Fig. 18a. Percent mortality of R. robini mites after
dipping in organophosphate compound
(inside each column % a.i.)

Fig. 18b. Percent mortality of R. robini mites after dipping in organophosphate compound (inside each column % a.i.).

Fig.19. Percent mortality of R. robini mites after dipping in carbamate compound
(inside each column % a.i.).

Fig. 20. Percent mortality of R. robini mites after dipping in various compounds

(inside each column % a.i.).

Fig. 21. Percent mortality of R. robini mites after dipping in synthetic pyrethroids (inside each column % a.i.).

4.3 Natural enemies

Little has been published on the natural enemies of Rhizoglyphus and their effect on pest populations. Andre (1941), Garman (1937) and Soliman et al. (1978) recorded some predaceous mites; the second author also a predaceous cecidomyiid gall midge (Lestodiplosis).

Natural enemies of both groups were likewise found in Israel. The rhodacarid mite, Protogamasellus sp. (determined by Prof. M. Costa, University of Haifa), was seen feeding, in large numbers, on Rhizoglyphus mites artificially introduced into the soil. The pests were present in such large numbers that the predator could not have had an important effect on their populations.

Heavily-infested garlic cloves, left in the field under open, shaded conditions were seen to be preyed upon by gall midge larvae. These were reared and sent to Mr. K. M. Harris (British Museum, Natural History, London), who identified them as Trisopsis sp. and Lestodiplosis sp. As Rhizoglyphus was exposed in a rather unnatural condition, the real significance of these gall midges is not known.

4.4 Discussion

All control efforts described in this chapter were aimed at reducing Rhizoglyphus damage in the field. Ascerno et al. (1981) recently reviewed methods of reducing mite numbers in greenhouse crops and reported on good Rhizoglyphus control in lily bulbs by soaking them in the acaricide dicofol (Kelthane).

The field data indicate that control measures are not always necessary. Plots known to be heavily infested - either because damage occurred there or through monitoring by traps - should be disinfected; solar heating appears to offer the best long-range option. Aldicarb and Phorate, although affording some short-term protection, are too toxic to be used on edible crops. DBCD was more promising.

A different approach is recommended in regard to fields where damage is first discovered during the growing season. In cases of light to moderate infestation, mite presence would probably only serve to thin out the plants, with no real effect on yields. In plots showing heavy damage chemicals will have to be applied. A range of pesticides is available (4.2.2), but the choice should depend on specific local conditions.

5. General discussion

5.1 The origin of Rhizoglyphus infestations in the soil

In Israel Rhizoglyphus appears to be a relatively new - or newly noticed - pest of onions and garlic in the field. Even if its damage had formerly been attributed to the onion maggot - as had indeed happened in the U.S.A. (Rawlins, 1955) - this still would not have explained severe, sudden outbreaks in fields left uncultivated for many

years. Any pest management program must consider the origin of the pest it is trying to manage. Keeping that pest out of susceptible crops may at times be the simplest and most effective policy. Hence our interest in the origin of Rhizoglyphus.

The most obvious theory, that the pest arrived in garlic cloves from formerly-infested fields, was not confirmed by summer-long examinations (2.3). Nor could the mites have been introduced by onion seeds, which are too small.

Observations that mite injury sometimes closely overlapped sites where manure had been applied (2.2.1, Fig. 3) suggested that Rhizoglyphus might have been introduced by this means. However, the mites were not found in manure (2.3), nor have they been reported from this habitat by others (i.e., Green, 1980).

The feeding experiments (3.2.2) showed Rhizoglyphus to be quite catholic in its diets; manures, especially of poultry source, which is richer in proteins, brought about large increases in mite numbers. But so did plant remains as well as macerated soil invertebrates. Furthermore, Rhizoglyphus has the capacity of moving down into the soil during hot and dry periods, surviving long periods under almost starvation conditions, and withstanding prolonged immersion in water. It is also a long-lived mite.

Taken in conjunction, these considerations lead us to postulate that Rhizoglyphus robini is a true soil-borne invertebrate. It has probably always lived in the soil, subsisting, rather marginally, on dead roots, animal remains, various molds and the like. As the soil began to be cultivated, i.e., irrigated and manured, Rhizoglyphus reacted rapidly by raising large populations. These mites prefer living plant material to dead and decomposing diets and are rapidly attracted to it. Therefore when onions or garlic were introduced to appropriate fields, the mites moved to feed on them, thus becoming pests.

It follows that no management practices can keep Rhizoglyphus out of certain areas; even disinfections could not eliminate the mite from the soil for more than a single season. Control should consist of containment efforts, aimed at reducing damage rather than reducing mite numbers. Pesticides should be left as the very last resort.

5.2 Guidelines for management of Rhizoglyphus

Most of the following discussion will concern the management of Rhizoglyphus populations in the soil; options for chemical control in bulbs and corms have been presented, among others, by Ascerno et al. (1981), Schread (1969) and Wilkin et al. (1976).

Firstly a distinction should be made between plots without any prior Rhizoglyphus history and those where the pest is already known to occur.

Results of pre-planting monitoring with traps would warn the grower of mite presence. Disinfection (solar heating is an important option) could then be undertaken. Another possibility - not explored in this project - is the incorporation of an appropriate pesticide in manure. This would also cancel the mite-encouraging properties of the manure. In any case, despite the risk of damage, there is no real cause for not growing onion and garlic in such hitherto-uninfested plots.

On the other hand, an effort should be made to avoid growing susceptible crops for at least two years on infested fields, unless soil disinfection is practiced. Thorough drying out during summer could well reduce Rhizoglyphus numbers to very low levels.

Another possibility is to use resistant or at least tolerant varieties (Section 2.2.3). This option has only been investigated in regard to garlic.

Not all mite infestations, even when followed by moderate die-back of plants in the field, should cause alarm. It was observed (2.2.1) that plots damaged in the autumn looked quite well in the spring, indicating that control measures would have been redundant. The lack of an appropriate threshold is of great hindrance here.

Chemical control measures should only be used in badly damaged fields, and, as suggested in the former paragraph, only during autumn. In spring, plants are stronger while mite populations are declining. The fact that even very few mites can cause plant death makes pesticide use even more precarious. However, several pesticides are available (4.2.2), should the decision be made.

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Evaluation of the Research Achievement With Respect to The Original Research Proposal

The following research topics were proposed in the original proposal:

1. A study of the biology of Rhizoglyphus in the laboratory, with emphasis on hypopus induction and termination.
2. Field survey for mite phenology, infestation extent and natural enemies, as well as possible crop resistance.
3. Development of sampling methods.
4. Relationships between Rhizoglyphus and various plant pathogens.
5. Cultural methods of mite control.
6. Chemical methods of mite control.

The biology of Rhizoglyphus was thoroughly studied and life tables were prepared (3.2.1). Hypopus induction was explored (3.2.2.2) as well as environmental factors causing its termination (3.2.2.2 and 3.2.3).

Mite phenology was followed by use of specially developed traps. Some estimates of infestation levels were obtained, as well as data on natural enemies and on one mechanism imparting tolerance to a garlic variety. The problem of sampling was tackled by means of the trapping; the enzymatic method may also be of value here.

The relationships between the mite and some pathogens were explored in regard to attraction, feeding and effect on hypopus biology.

Cultural (solar heating) and chemical modes of control were tried and some were found to be better than others.

In addition, the following subjects were studied:

1. The effect of various natural diets (manures, roots, soil, invertebrates) on mite increase.
2. Mite survival while immersed in water as well as under cool conditions.
3. The physical and chemical composition of some soils in which Rhizoglyphus occurred.
4. Nutritionally-induced variations in the taxonomically-important sc i setae.

The total sum of the obtained data allowed us to postulate on the origin of Rhizoglyphus as an outdoors pest and to formulate guidelines for its management in the field.

Description and Results of Cooperation

Studies done in Israel and in the U.S. on basic biology, field ecology and mite rearing show that the mites in both areas behaved similarly to environmental stimuli. Rhizoglyphus was a problem and attacked agricultural crops under similar commercial conditions in both regions. Mites were associated with underground plant storage organs - bulbs, tubers, rhizomes, corms - that showed symptoms of attack by soil fungi. The latter occurred readily under moist soil conditions, and so mite outbreaks appeared worse under high soil moisture. Mite populations appeared to survive in field soils by moving deeper into the soil in response to lower moisture levels and lower temperature. Hypopodes formed in populations of both countries as a facultative stage; no specific environmental or dietary component was demonstrated to have an overwhelming influence on the stage. Hypopode transformation occurred at low levels under conditions of favorable temperature and humidity, but was greatly enhanced by addition of a source of nutrition.

Thesis

1. Capua, S. 1981. A contribution to the biology of the soil mite Rhizoglyphus robini Claparede.

Submitted to the Faculty of Agriculture, The Hebrew University of Jerusalem.

Supervisor: Prof. U. Gerson.

BENEFIT TO AGRICULTURE

This project was intended to study the biology and ecology of bulb mites in order to supply knowledge required for their management.

The trapping method would be of benefit as it allows growers and extension personnel to monitor fields prior to or during the growth period of susceptible crops. This will place decision making on a much sounder basis.

Observations on the causes of Brazilian garlic tolerance to the mite could also be applied to other affected crops and possibly to other pests.

Observations that even severe damage in the fall does not necessarily lead to stand reductions in the spring should alleviate growers' fears.

The enzymatic method developed for separating mites from plant tissue could facilitate further studies on chemical control.

Our theory on the mechanism of outbreak of mite populations in the field could also be applicable to other soil pests.

The pre- and post-planting control tactics devised could be of much benefit should they be needed.

And finally, the management guidelines set down should enable growers and extension personnel to coexist with the mite without excessive resort to pesticides.

List of Published or Accepted Manuscripts

1. Gerson, U., S. Yathom and J. Katan. 1981. A demonstration of bulb mite control by solar heating of the soil. - *Phytoparasitica* 9: 153-155.
2. Gerson, U. and D. Thorens. 1982. Mating frequency as an indication of food quality for the bulb mite, Rhizoglyphus robini Claparede. - *International Journal of Invertebrate Reproduction* 5: 201-206.
3. Gerson, U. and S. Capua. 1982. Allometric variation in Rhizoglyphus robini Claparede (Acari: Astigmata: Acaridae). - *Israel Journal of Entomology* 16: 69-72.
4. Capua, S. and U. Gerson. The effects of humidity and temperature on hypopodial molting of Rhizoglyphus robini. - *Entomologia et Applicata*, in press.
5. Gerson, U., S. Capua and D. Thorens. Life history and life tables of Rhizoglyphus robini Claparede (Acari: Astigmata: Acaridae). - *Acarologia*, in press.