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Life History Parameters of the Two-spotted Spider Mite (*Tetranychus urticae* Koch) Feeding on Bean Leaves Treated with Pyrrolizidine Alkaloids

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Key words: pyrrolizidine alkaloids; *Lithospermum canescens*; *Boraginaceae*; Indian paint; hoary puccoon; *Tetranychus urticae*.

In this study we assess the effect of pyrrolizidine alkaloids (PAs) extracted from *Lithospermum canescens* on the biology of the two-spotted spider mite (*Tetranychus urticae Koch*). *Lithospermum canenscens* (Michaux) Lehm. (*Boraginaceae*) is a common prairie plant also known as Indian paint or hoary puccoon. A mixture of seven PAs with known chemical structures was used in this investigation. Mites treated with PAs showed a high mortality of juveniles, a decrease in female fecundity and a shortened longevity. The intrinsic rate of population increase (r_m) was used as an indicator of *T. urticae* population performance after treatment with PAs. The r_m value obtained with alkaloid-treated leaves was lower than that for mites developing on untreated leaves, which indicates that the mite population would develop much slower on treated plants. The results suggest that further studies should be performed to assess the possible use of PA extracts for spider mite control.

INTRODUCTION

The two-spotted spider mite (*Tetranychus urticae* Koch) is a common pest attacking cultivated plants, including a number of medicinal herbs. Injury caused by *T. urticae* is very severe and nowadays most fields are treated with acaricides such as clofentezine, hexythiazox, fenbutain oxide and tetradifon to control the pest (DeAngelis *et al.*, 1983; Morris *et al.*, 1996). To find an alternative control method we assessed the effect of pyrrolizidine alkaloids (PAs) extracted from *Lithospermum canescens* on the biology of the two-spotted spider mite. *Lithospermum canenscens* (Michaux) Lehm. belongs to the *Boraginaceae* family. It is a common prairie plant also known as Indian paint or hoary puccoon (Forey, 1991).

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So far, studies on the application of plant-derived chemicals against the two-spotted spider mite have been limited to tests of the antifeedant activity and toxicity of azadirachtin, a steroid-like tetranortriterpenoid isolated from the Indian neem tree (*Azadirachta indica*). The neem extracts affect the arthropod pests in different ways. They can act as growth regulators to prevent the moulting of larvae, to inhibit egg development or the process of pupation and to disrupt mating and they have a repellent and antifeedant effect on pests (Mansour *et al.*, 1987; Schmutter, 1990; Mansour and Ascher, 1995; Gripwall, 1999). The influence of neem extracts on *T. urticae* was tested in several experiments. Increased mortality of both adults and juvenile stages and a lower female fecundity were observed. The effect of several herb extracts — *Achillea millefolium*, *Taraxacum officinale*, *Matricaria chamomilla* and *Salvia officinalis* — on mite biology was examined (Tomczyk and Szymańska, 1995; Kawka and Tomczyk, 2002). These authors demonstrated strong inhibition of the feeding activity of mites (by 28–35%) after treatment with sage extracts. In our previous study (Furmanowa *et al.*, 2001, 2002) we found that *Taxus* needle extracts adversely affected most biological parameters responsible for mite population increase. They prolonged the duration of development, the mortality of developmental stages was increased and the fecundity of females was reduced by 60–70%.

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MATERIAL AND METHODS

Experiments were conducted under laboratory conditions. *Tetranychus urticae* used in this study originated from laboratory culture reared on dwarf French beans (*Phaseolus vulgaris* L.).

Experimental unit

The leaf arena technique was used in this study. Bean leaves were placed on a water-saturated bed. The lower leaf surface was used for the experiment. The units were held in an environmental chamber produced by Sanyo Electric Trading Co., Ltd at 23 ± 1 °C, 60% relative humidity and a 16/8 h light/dark cycle.

Experimental procedure

The experiment started with 100 leaves dipped in the test solution of 17 mg of PAs, 2 ml of methanol and 168 ml of distilled water (1.15 μg PAs cm^{-2} leaf). Another 100 bean leaves were treated with a solution of 2 ml of methanol and 168 ml of distilled water. Individual females were placed on the leaves and allowed to lay only one egg each and then were removed before further oviposition. The development of eggs and juvenile stages was followed every 24 h. The length of developmental time from deposition of the eggs until adult emergence and juvenile mortality was measured. When adults appeared they were sexed and counted. The sex ratio was calculated for the progeny of mated females because the two-spotted spider mite is an arrhenotokous parthenogenic species and unmated females produce only males.

In the experiment measuring the rate of reproduction and longevity of *T. urticae*, 50 female deutonymphs were sampled for each of two trials. Males were introduced for insemination of the females immediately after eclosion. The initial number of mite pairs in the experiment was 100. Moreover, the use of detached leaf cultures in experiments is always connected with the loss of some specimens that die of unnatural causes, e.g. drowning in the water-soaked tissue. In this experiment 66 pairs of mites could be selected to calculate life history parameters. Each pair was maintained on a separate freshly treated leaf. Total fecundity was determined by daily counting and removing eggs laid by each female during the whole period of its life. Mites were transferred to fresh treated leaves every 4–5 days. Observation was conducted until the last female died. The significance of the difference in the medians was analysed using the Kruskal–Wallis test.

The intrinsic rate of increase r_m can be calculated from the obtained life history components of *T. urticae*, such as the developmental rate, the ovipositional rate, the survival rate and the proportion of females in the offspring. The intrinsic rate of increase was defined by Birch (1948) as the actual rate of increase of the population under specified constant environmental conditions in which space and food are unlimited and when there are no mortality factors other than physiological ones. The following values are taken from life history data to construct the life table: x is the age of individuals in days; l_x is the number of individuals alive at age 'x' as a proportion of one; m_x is the number of female offspring produced per female in the age interval 'x'; and s_x is the proportion of daughters in the offspring of females.

The intrinsic rate of increase was calculated from the data in the life table according to the formula:

$$\sum e^{-rmx} l_x m_x s_x = 1$$

where 'e' is the base of natural logarithms.

The net reproductive rate (the net rate of increase per generation) is:

$$R_0 = \sum_{x=1}^{\infty} l_x m_x$$

The generation time (the mean time between parents and offspring) is:

$$T = \frac{\sum (x l_x m_x)}{R_0}$$

and the finite rate of increase $\lambda = e^{rm}$.

Pyrrolizidine alkaloid extract

In the present investigation the purified total extract of PA fraction was used in the test on *T. urticae* Koch. The extract was prepared from *Lithospermum canescens* (*Boraginaceae*) plant material collected in June 2001 in Togo, Canada. Extraction of dried plant material (10 g) with methanol (200 ml) was carried out in a Soxhlet apparatus for 24 h and then the solvent was evaporated from the extract solution under reduced pressure at 40 °C. The dry residue was purified using methylene chloride and ethylic ether, separately, at pH 2. The total PA fraction was isolated from the extract by shaking twice with methylene chloride at pH 9, salting out the water phase after the first stage. Thin-layer chromatography (silica gel F_{254}, CH_2Cl_2–CH_3OH–NH_4OH (25%), 85 : 14 : 1) and Mattock's reaction were used for confirming the presence of PAs in the extract. This method was described earlier by Roeder and Wiedenfeld (1977) and Wiedenfeld and Roeder (1979). The composition of the tested extract is known from earlier studies of the same plant material of this species by Wiedenfeld *et al.* (in press). These authors isolated and elucidated, by spectroscopic methods, the structures of seven PAs from *L. canescens*. Besides the already known lycopsamine, O^7-acetyllycopsamine and O^7-acetylintermedine, four new PAs were found: O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine (i.e. O^7-3-hydroxy-3-methyl-butanoyl)-rinderine), O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(−)-viridifloryl-heliotridine (i.e. O^7-3-hydroxy-3-methyl-butanoyl)-echinatine), O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridin and O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(−)-viridifloryl-heliotridine, respectively.

RESULTS AND DISCUSSION

Table 1 shows the duration of development, mortality during development, fecundity and longevity of females in both treatments. There was no significant difference

Table 1—Selected life history components of *Tetranychus urticae* feeding on bean leaves treated with pyrrolizidine alkaloids (1.15 μg cm^{-2} leaf) from *Lithospermum canescens*

Studied parameter	Treated leaves	Untreated leaves
Developmental time (days) ± SEM	11.91 ± 0.23 (NS)	11.07 ± 0.14 (NS)
Mortality (%)	22	8
Proportion of females	0.51	0.54
Total fecundity (eggs/per female) ± SEM	32.73 ± 0.97*	80.21 ± 1.73*
Longevity (days) ± SEM	13.54 ± 0.42*	23.78 ± 0.29*

NS, not significant; * $P < 0.05$, Kruskal–Wallis test.

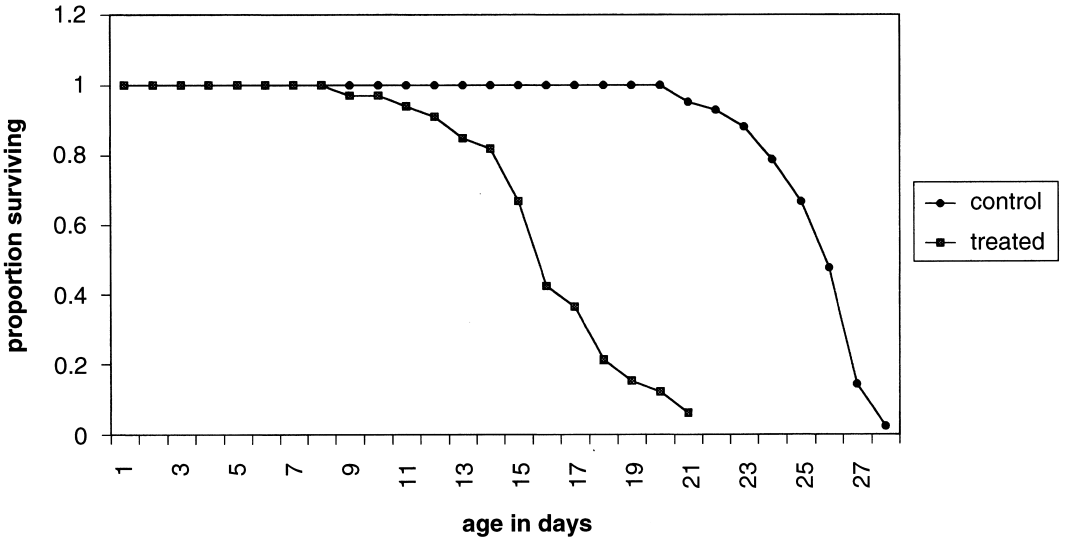

Figure 1. Longevity of *Tetranychus urticae* females on bean leaves treated with pyrrolizidine alkaloids.

in the duration of developmental time among mites feeding on leaves untreated and treated with alkaloids but the overall survival of *T. urticae* juveniles on the treated leaves was much lower compared with the control. The number of females obtained in both treatments was slightly higher than that of males.

The course of l_x curves illustrating the age-specific survival was similar for both groups of females during the first 10 days of their life. Sixty per cent mortality occurred on the 17th day in the group of females feeding on treated leaves. On day 23 no female was alive. In contrast, mites feeding on the control leaves lived longer because on the 26th day 50% of them were still alive (Fig. 1).

Oviposition started on the second day after adult emergence. Daily fecundity was at its highest during 5–14 days of reproduction. Total fecundity on the leaves treated with PAs was much lower than in the control group. The ovipositional period for females feeding on untreated leaves lasted 26 days whereas for those on treated leaves it was 21 days (Fig. 2).

The population parameters of *T. urticae* feeding on PA-treated leaves are shown in Table 2. The r_m value obtained with PA-treated leaves was lower than that for females in the control group. Because a very similar generation time was obtained for both tested groups of females, the net reproduction rate can be compared. The mite population during one generation increased by only 13.01 on treated leaves but by 39.85 in the control group.

The parameters showed a similarity to those obtained

Table 2—Effect of pyrrolizidine alkaloids on population parameters of *Tetranychus urticae* females

Parameter	Treated leaves	Untreated leaves
Intrinsic rate of increase (r_m)	0.134	0.182
Net reproductive rate (R_0)	13.01	39.85
Generation time (T)	20.14	19.10
Finite rate of increase (λ)	1.14	1.20

for *T. urticae* developing on leaves treated with yew extract (Furmanowa *et al.*, 2001, 2002). Mites treated with extracts either from yew needles or PAs showed a high mortality of juveniles, a decrease in female fecundity and a shortened longevity. The finite rate of increase was ca. 1.1, compared with 1.2 in the control group, which indicates that the mite population would develop much slower on PA-treated leaves than on untreated plants. The higher mortality of *T. urticae* juveniles and lower fecundity of females on leaves treated with extracts could be the result of their lower consumption rate and a direct toxic effect of the extract. These results correspond to the data reviewed by Mattocks (1986), indicating that PAs mostly act as feeding deterrents or can be transformed into toxic pyrrolic metabolites.

To clarify the mechanism of PA action on two-spotted spider mites, further studies are needed. The results obtained in the present study showed that PA extract

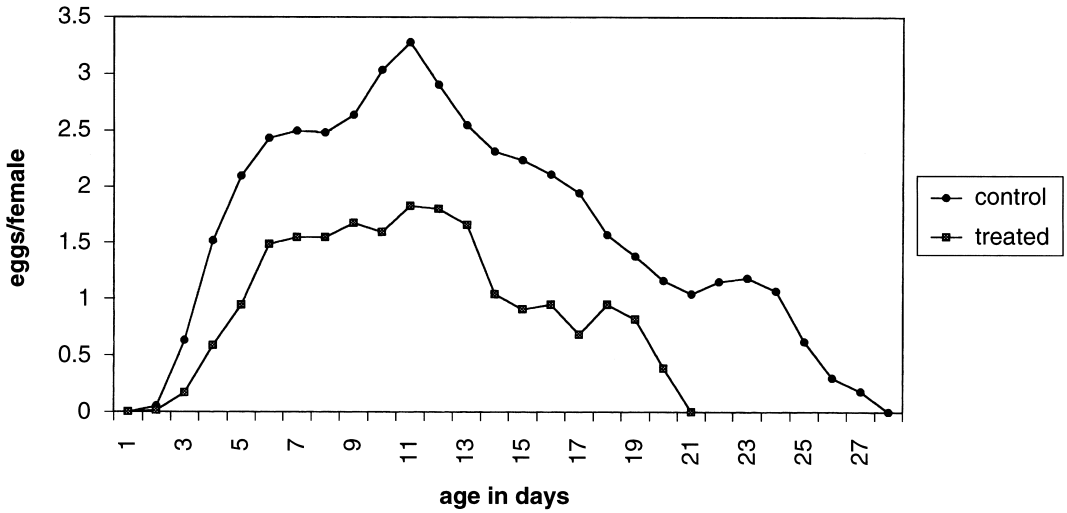

Figure 2. Age-specific fecundity of *Tetranychus urticae* females feeding on bean leaves treated with pyrrolizidine alkaloids.

had a significant impact on the biological parameters of the two-spotted spider mite. As a result, the development of mite populations was slower than on untreated plants. The results of our study indicate that PA extract can be useful in controlling spider mite populations on ornamental plants, which would be of practical value.

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