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SENITA CACTUS ALKALOIDS: THEIR SIGNIFICANCE TO SONORAN DESERT *DROSOPHILA* ECOLOGY*

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Abstract—The toxicity of senita cactus (*Lophocereus schotti*), and the alkaloids derived from it, pilocereine (I) and lophocereine (II), have been tested against *Drosophila pachea* and eight other species of *Drosophila* of the Sonoran Desert. The cactus and pilocereine kill the adults and/or progeny of all species but *D. pachea*. The alkaloid is probably the main reason why no other species than *D. pachea* breeds in the rotting stems of senita cactus.

INTRODUCTION

THE SOLE breeding site occupied by *Drosophila pachea* in the Sonoran Desert is the rotting stem of the senita cactus, *Lophocereus schottii*, where it obtains a sterol, schottenol, necessary for growth and reproduction. Other species of desert-adapted *Drosophila* do not breed in stems of senita because it contains materials that are toxic to them. Incorporation of the cactus (or a sterol derived from it) into the medium is mandatory for maintenance of *D. pachea* in the laboratory. Incorporation of the cactus (but not the sterols derived from it) into the medium of other species of *Drosophila* is toxic in varying degrees to the adults and larvae (HEED and KIRCHER, 1965; HEED and JENSEN, 1966).

We suspected the alkaloids in senita cactus to be the toxic principles. The dry cactus contains 3·7% crude alkaloid (DJERASSI *et al.*, 1953), at least 0·6% pilocereine (I) (DJERASSI *et al.*, 1962), and, at most, 0·18% lophocereine (II) (DJERASSI *et al.*, 1958) (Fig. 1). Initial experiments with senita alkaloid mixture, pilocereine, and impure lophocereine on several desert-adapted species of *Drosophila* showed that these materials were toxic in varying degrees (GROVE, 1965). The work reported here deals with more species, longer tests, and the use of pure lophocereine.

MATERIALS AND METHODS

Drosophila colonies were maintained in the laboratory in shell vials on regular banana medium. The medium for *D. pachea* was supplemented with senita cactus or Δ^7-stigmasten-3β-ol (schottenol) (HEED and KIRCHER, 1965).

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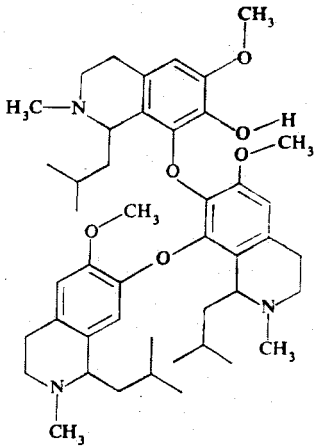

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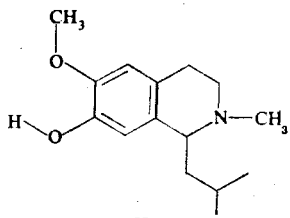

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FIG. 1. Structure of the cactus alkaloids pilocereine (I) and lophocereine (II).

Alkaloids

Pilocereine (I) was isolated from senita cactus and purified by crystallization from ethyl acetate, m.p. and m.m.p. 171·5 to 172·5 °C; same mobility on TLC and i.r. spectrum as an authentic sample.* Lophocereine (II) was synthesized by the method of BOBBITT and CHOU (1959), purified by distillation at 0·05 mm, bath temperature 140 to 170°C and a picrate was prepared, m.p. 190 to 191·5°C (lit. m.p. 191·5–193°C, BOBBITT and CHOU, 1959).

Alkaloid tests with Drosophila

For tests with species other than *D. pachea*, alkaloids I and II were added to regular laboratory medium (bananas–yeast–agar–corn syrup–malt extract) at a concentration of 1% of the dry ingredients in the diet. The individual alkaloids (260 mg) were dissolved in propionic acid (1·25 ml), diluted to 10 ml with water and added to 300 ml of freshly prepared medium (13 mg alkaloid/15 ml medium per vial). The control and senita–cactus-supplemented vials (3·5 g autoclaved cactus per 15 ml medium—roughly 13 mg crude alkaloid mixture) contained the same amount of propionic acid to retard the growth of micro-organisms.

For *D. pachea* tests the medium was supplemented with senita cactus as above to contribute the necessary sterol. Increasing quantities of the two alkaloids in propionic acid were added to this medium to test the tolerance of *pachea* to the two compounds.

Fifteen males and fifteen females of each species were aged 7–14 days and tested on the alkaloid-containing media in duplicate vials. Vials that contained the regular laboratory medium and medium plus senita cactus were run concurrently as controls. Adult mortality in each vial was checked up to 30 days; during this time the remaining adults were transferred once to fresh vials to avoid overlap

* We thank Dr. CARL DJERASSI for providing us with this sample of pilocereine.

of generations. The number of adult progeny in each vial was counted and their viability checked on the same type of medium from which they emerged.

RESULTS AND DISCUSSION

The toxicity of pilocereine (I) and senita cactus toward the adults of eight species of *Drosophila* that inhabit the Sonoran Desert is shown in Fig. 2. In most cases the alkaloid toxicity paralleled or was more effective than that of the cactus. In only one case (*D. nigrospiracula*) was the cactus more toxic than the alkaloid, and in this case, the latter appeared to have little effect on adult longevity when compared to the controls.

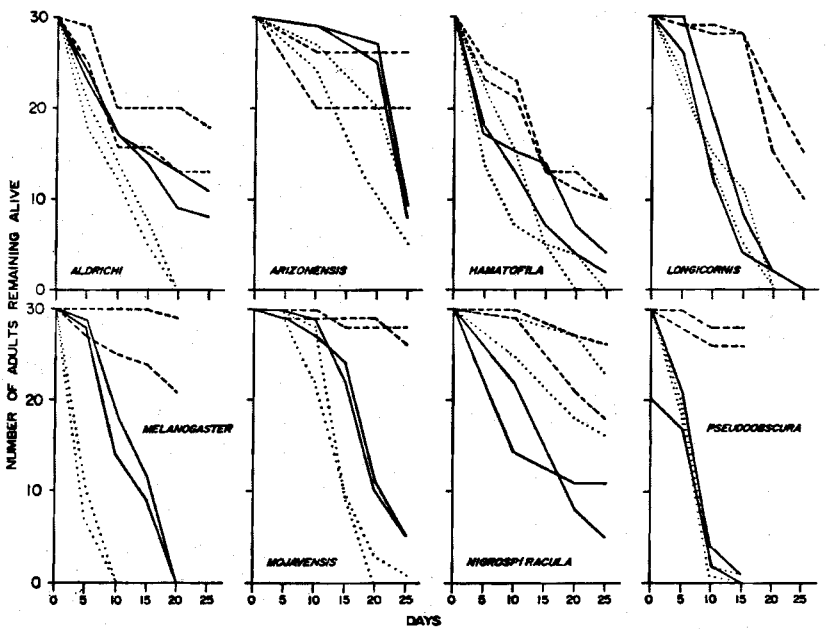

FIG. 2. Adult mortality of eight species of *Drosophila* on regular, senita cactus and pilocereine media (1% alkaloid). ----, Control; —— senita; ····, pilocereine.

A similar experiment with lophocereine (II) showed that except for *D. hamatofila* (control 19,26; alkaloid 0,6 adults remaining alive after 30 days) this alkaloid had essentially no effect on adult longevity when it represents 1 per cent of the diet. Since this concentration is considerably higher than the concentration of lophocereine in senita cactus (<0·18%), the alkaloid does not contribute much to the toxicity of the cactus to adult *Drosophila* in nature. Pilocereine (I), the trimer of lophocereine (II), is quite stable to air over long periods of time, whereas lophocereine darkens in 1 day in air at room temperature. This lability of lophocereine may enable rapid detoxification of the alkaloid by the insects; a process which may

also occur in the medium during the period of the tests and in the cactus as it is rotted by micro-organisms.

The toxicity of senita cactus and the two alkaloids to the larvae of the various species is shown in Table 1. All were affected by senita and pilocereine; either the larvae died in the first instar or the F_1 adults died before producing progeny or the number of F_1 and F_2 were drastically reduced when compared to controls. The toxicity of the alkaloid again was similar to that of the cactus. The results with lophocereine show a diminished toxicity toward the various species. Four of the eight species were not greatly affected by the alkaloid; the F_1 matured and produced progeny little different from the controls. Of the other four species, *D. aldrichi* and *D. nigrospiracula* yielded F_1 that died before reproducing and *D. hamatofila* and *D. longicornis* produced essentially no F_1. The latter four species are difficult to rear on normal banana media; they are best maintained on media supplemented with prickly pear cactus.

TABLE 1—NUMBER OF PROGENY AND THEIR VIABILITY FROM FIFTEEN MALES AND FIFTEEN FEMALES OF EIGHT SPECIES OF SONORAN *Drosophila* REARED ON FOUR MEDIA

Species	Control, F_1 adults (a)	Control, F_1 adults (b)	Senita cactus F_1 adults	Senita cactus F_2	1% Pilocereine F_1 adults	1% Pilocereine F_2	1% Lophocereine F_1 adults	1% Lophocereine F_2
D. aldrichi	190	300	0*	0	0*	0	67†	0
D. arizonensis	629 +	974	542‡	0	269‡	10‡	570	+
D. hamatofila	210	642	0	0	0	0	5	—
D. longicornis	0§	558	167‡	0	0*	0	0	0
D. melanogaster	938	1359	249	+ +‖	101	0*	826	+
D. mojavensis	812 +	1577	500 + ‡	41	150‡	10‡	880	+
D. nigrospiracula	0§	46	40‡	+	30‡	0	26†	0
D. pseudoobscura	372 +	782	263	+	173	32‡	778	+

(a) Controls for senita cactus and pilocereine tests. (b) Controls for lophocereine test. * Died in first instar. † Died, not fertile. ‡ Adults died early. § Did not go well because of lack of prickly pear cactus. ‖ Many pupae.

An unexpected result was the absence of adult mortality when *D. pachea* was placed on media that contained up to 14% pilocereine or 10% lophocereine in addition to 1% senita alkaloids (Table 2). In the same period of time (20 days) 1% pilocereine killed all of the adult *D. aldrichi, D. hamtofila, D. longicornis, D. melanogaster, D. mojavensis,* and *D. pseudoobscura*; only *D. nigrospiracula* adults were relatively unaffected by this alkaloid (Fig. 2). The higher concentrations of pilocereine diminished the number of progeny produced by *D. pachea*, yet in all cases they were fertile when tested on regular medium supplemented with senita cactus. The higher concentrations of lophocereine (5 and 10%) produced a diminution in the number of *D. pachea* F_1 approximately equal to that of 4% pilocereine (Table 2), again demonstrating the higher toxicity of pilocereine.

TABLE 2—ADULT SURVIVAL AND NUMBER OF PROGENY PRODUCED WHEN ADULT *D. pachea* WERE PLACED ON MEDIA CONTAINING INCREASING AMOUNTS OF PILOCEREINE (I) AND LOPHOCEREINE (II)

Concentration of supplemented alkaloid %	Pilocereine test				Lophocereine test			
	Number of live adults after 20 days		Total number of progeny after 6 wk		Number of live adults after 20 days		Total number of progeny after 6 wk	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
0 (senita only)	27	29	441	454	27	25	315	*
1	30	27	345	156	17	25	98	25
2	—	—	—	—	21	16	180	210
4	28	27	69	127	—	—	—	—
5	—	—	—	—	7	17	68	87
9	28	26	8	3	—	—	—	—
10	—	—	—	—	23	23	32	74
14	24	18	2	7	—	—	—	—

All vials contained senita cactus equivalent to 1% crude alkaloid (a), (b) Duplicate vials.
* Moulded over.

In this instance, however, a significant number of dead (brown) eggs were observed at the 5 and 10% levels and only a few dead eggs were apparent in vials that contained 2% lophocereine. The compound may pass into the eggs when *D. pachea* females feed and affect hatchability. The higher concentrations of pilocereine killed *D. pachea* larvae mostly during the third larval instar. The killing effect was dramatic since many active larvae died within a 48 hr period.

The Cactaceae are known for their high alkaloid content. These compounds are generally of the β-phenylethylamine or tetrahydroisoquinoline type derived from aromatic amino acids (RETI, 1950, 1954). Pilocereine (I) is distinct from previously known cactus alkaloids not only by its large size, but also by the 1-isobutyl group on each of the tetrahydroisoquinoline structures (DJERASSI *et al.*, 1957). Tetrahydroisoquinoline alkaloids in general have marked physiological effects (RETI, 1950, 1954). The pharmacological studies done with pilocereine show it to be toxic to rats, have diverse effects on the blood pressure of dogs, cats, rats, and chickens, and to be about as effective as quinine against malaria in canaries (POWELL and CHEN, 1956). It also generally relaxes smooth muscle and may affect the insects' gut in this way and not enable them to metabolize their food. Experiments with the alkaloid and a dye in the medium showed that the adult *Drosophila* were ingesting the food and did not die of starvation.

The toxicity of alkaloids to insects (especially of nicotine and its analogues) has a long history (SHEPARD, 1951; METCALFE, 1955; BORKOVEC, 1966; CROSBY, 1966). Recent work has shown that demissin and tomatin, alkaloid glycosides in Solanaceae, are gustatory inhibitors to the Colorado potato beetle (SCHREIBER, 1959) and that sparteine appears to be a feeding stimulant for aphids on broom

(SMITH, 1966). Our work, however, is the first to demonstrate the species specific effect of an alkaloid and to relate it to the insect ecology of a region.

The ability of *D. pachea* to tolerate the alkaloid pilocereine present in the stems of senita cactus together with its absolute requirement for shottenol explains this unique cactus–*Drosophila* relationship in the Sonoran Desert. Other species of *Drosophila*, which could also utilize the cactus sterols, are prevented from breeding in the plant because of the toxicity of pilocereine to the adults and larvae.

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