# OBSERVATIONS ON THE PITCHER LIQUOR OF THE SARRACENIACEÆ

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## Volume of the Normal Pitcher Secretion

The pitchers of all North American *Sarraceniaceæ* grow to a considerable size and even approach their full size before the orifice of the pitcher opens. These hermetically sealed or closed pitchers normally contain a larger or smaller volume of clear fluid which is transparent and colorless, though sometimes rendered slightly milky by suspended particles. The volume of the fluid or pitcher liquor is much greater in large vigorous pitchers than in small stunted ones in which the liquor may be almost entirely absent. In *Sarracenia purpurea* and *S. psittacina*, the quantity of pitcher liquor is very small; even in the larger pitchers, it usually appears only as a studding of minute perspiration-like drops on the lower wall and rarely collects in the bottom of the cavity. In all the other species, the pitcher liquor occurs in sufficient quantity to be drawn off with a pipette, and may fill the narrow tube to a height of several inches in vigorous pitchers.

After opening, the pitchers of *Sarracenia purpurea* usually become filled, entirely or partly, with rain water. Certain species, especially *Sarracenia purpurea* and *S. psittacina*, may be partially or completely submerged in time of flood as a result of their shape, size, and habitat. Beating rains may enter, in greater or less degree, the pitchers of the remaining species of *Sarracenia*. The level of the liquid contents of the pitcher, thus augmented, may be reduced again by evaporation or absorption. In dry weather, and especially when the soil about the roots becomes dry, the pitchers may be found to have lost much or all of their liquid contents. Under these varying circumstances, the contents of an open pitcher may consist principally of a mass of insect remains ranging from dry and friable to moist and broth-like, or of insect cadavers bathed in a liquid which may range from clear to markedly turbid, or of insect captures floating in an excess of liquid (rain water or flood water) which, necessarily, highly dilutes the secretion of the pitcher.

The volume of the pitcher liquor, reported in Table I for *Darlingtonia*

*californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii* are based on measurements of the volume present in normal, full-sized pitchers—closed, plugged, or open—of these species growing in favorable localities at the height of their season and not materially affected by external conditions. The volumes recorded represent the amount of secreted liquid present in the pitchers at the time of examination. *Closed* pitchers were used, as nearly as possible, at their point of opening. *Plugged* pitchers, in age and development, were open and active but free from insect captures. The top of a closed pitcher, which was about to open, was folded over; a piece of paper bearing the date was included in the fold; and one or more pins were passed through the doubled pitcher and paper, effectually sealing the top. The pitcher did not wither, but continued to grow and to expand its top even above the fold; it was permitted to remain in this condition for 1 week in the case of the Sarracenias and for as long as 1 month in the case of *Darlingtonia californica*. *Open* pitchers were fully mature, and actively engaged in the capture of insects.

*Determination of the volume.*—The upper portion of the pitcher was removed with scissors until the pitcher liquor was within reach of a plain pipette, the lower portion of which was of fine bore. As each portion of the liquor was removed by means of the pipette, another section of the pitcher was cut off. This procedure was repeated until the entire liquid contents of the pitcher had been collected. The total liquid contents of a definite number of pitchers were collected in a stoppered vial by transfer from the pipette. The total volume obtained from the lot was measured in a narrow graduated cylinder; and the average volume per pitcher was calculated.

Not many pitchers of *Sarracenia rubra* were obtainable. The pitchers of *Sarracenia minor* were of a size far below the recorded maximum for this species. Therefore quantitative measurements of the volume of liquor present in the pitchers of these two species have not been reported. No records are included for *Sarracenia psittacina* since the pitchers of this species do not secrete liquor in measurable quantity during their seasonal history. The liquid ordinarily present in open pitchers of *Sarracenia purpurea* is the secretion of the pitcher highly diluted by rain water, for the shape of the pitcher of this species readily permits the entrance of falling rain; therefore no measurements were made of the volume of liquor present in such pitchers. The amount of liquor present in closed pitchers of this species is so slight that its volume is not measureable.

Observations and measurements made on many individual pitchers, as well as the general results presented in Table I, indicate that the pitcher liquor progressively increases in volume as the pitchers develop. A further marked increase in the volume of the pitcher liquor occurs after the lid and lips have opened, and a still further increase is usually apparent after the capture of prey.

On account of the shape of the pitchers and the habitat of the plant, the volume of the pitcher liquor in *Darlingtonia californica* is less influenced by external conditions than in the Sarracenias. The progressive increase in the volume of the pitcher liquor is shown by the following data. As reported in Table I, 82 *plugged* pitchers, studied in 4 lots during June and July, contained, on the average, 2.50 cc. of liquor per pitcher. A single lot of 25 pitchers, plugged in June and studied during the following September, in the same bog as the other lots, contained, on the average, 3.38 cc. of liquor per pitcher. As reported in Table I, 161 *open* pitchers, examined in 5 lots during June and

TABLE I.—AVERAGE VOLUME OF THE LIQUOR IN THE PITCHERS OF CERTAIN SARRACENIACEÆ.

| | *Closed Pitchers* | | | *Plugged Pitchers* | | | *Open Pitchers* | | |
|---|---|---|---|---|---|---|---|---|---|
| *Genus and Species.* | *Number of lots measured.* | *Total number of pitchers.* | *Average volume per pitcher, cc.* | *Number of lots measured.* | *Total number of pitchers.* | *Average volume per pitcher, cc.* | *Number of lots measured.* | *Total number of pitchers.* | *Average volume per pitcher, cc.* |
| *Darlingtonia californica* | 10 | 353 | 1.08 | 4 | 82 | 2.50 | 5 | 161 | 2.80 |
| *Sarracenia Sledgei* | 4 | 325 | 0.25 | 2 | 31 | 0.38 | 3 | 74 | 0.38 |
| *Sarracenia flava* | 6 | 566 | 0.66 | 1 | 8 | 1.30 | 4 | 160 | 1.50 |
| *Sarracenia Drummondii* | 4 | 230 | 0.44 | 1 | 20 | 0.66 | 4 | 138 | 0.58 |

July, contained, on the average, 2.80 cc. of liquor per pitcher. A single lot of 25 open pitchers, examined during the following September in the same bog as the other lots, contained, on the average, 4.04 cc. of liquor per pitcher.

The volume of pitcher liquor varies widely in any given species with the size, vigor, and degree of development of the pitcher. Thus in the 10 lots of *closed* pitchers of *Darlingtonia californica*, which were strictly comparable with each other, the maximum average volume of pitcher liquor for any lot was 1.60 cc. per pitcher, while the general average for all 10 lots was 1.08 cc. per pitcher. Also in the 5 lots of *open* pitchers of this species, likewise strictly comparable with each other, the maximum average volume of pitcher liquor for any lot was 3.61 cc. per pitcher, while the general average for all 5 lots was

2.80 cc. per pitcher. Individual pitchers of unusual size or vigor frequently contained pitcher liquor far in excess of the maximum average volumes just reported.

### Reaction of the Pitcher Liquor

The reaction of the pitcher liquor to litmus was determined for the species mentioned below, using liquor from individual pitchers and composite samples of liquor from many pitchers. The mode of collection of the pitcher liquor, described above, yielded a liquid uncontaminated by the plasma of the pitcher tissues. Blue and red litmus paper (Squibb) was used; this reagent is sensitive to 0.004 normal acid and alkali respectively.

TABLE II.—REACTION OF THE PITCHER LIQUOR TO LITMUS

| *Genus and Species.* | *Closed Pitchers.* | *Plugged Pitchers.* | *Open Pitchers.* |
|---|---|---|---|
| *Darlingtonia californica* | neutral | neutral | neutral |
| *Sarracenia minor* | neutral | | neutral to faintly acid |
| *Sarracenia Sledgei* | acid | usually neutral | neutral |
| *Sarracenia flava* | acid | | neutral to faintly acid |
| *Sarracenia Drummondii* | markedly acid | usually neutral | neutral |
| *Sarracenia rubra* | slightly acid | | |

The reaction of the pitcher liquor showed variations in the individual plugged pitchers of two species. In the case of *Sarracenia Sledgei*, the liquor was neutral in 20 pitchers, acid in 3 pitchers, and faintly alkaline in 2 pitchers. In the case of *Sarracenia Drummondii*, the liquor was neutral in 13 pitchers, acid in 5 pitchers, and faintly alkaline in 2 pitchers.

The change from an acid to a neutral reaction, which occurs in the pitcher liquor of several species upon the opening of the pitchers, does not depend upon or result from the capture of prey, for the same change occurs in plugged pitchers from which insects are carefully excluded.

### Mechanical Stimulation

In a series of experiments on mechanical stimulation of the pitchers, efforts were made to simulate the struggles of entrapped insects by dangling strings of glass beads, gentle scratching with elastic wire brushes, etc. These experiments were made on *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*, using pitchers of all ages, closed, just open, and open active pitchers both with and without prey. The period and degree of stimulation and also the elapsed time between stimulation and withdrawal of the pitcher contents were varied. However, no measurable effect upon the volume of the pitcher liquor was detected in any of the experiments.

### Food Stimulation

Reference has been made to the work of Mrs. Austin[7] on the response of *Darlingtonia californica* to food stimulation. An account of many additional experiments on such stimulation is given by her in her unpublished letters and manuscript journal, which are preserved among the Canby Papers deposited in the library of the Society of Natural History of Delaware at Wilmington.

In our experiments, use has been made of milk, beef broth, meat, other foods of animal origin, and inorganic salts.

*Milk.*—Fresh skim milk was diluted with its own volume of water, sterilized by heat, and permitted to cool to the temperature of the atmosphere. Measured volumes of this milk were introduced into the pitchers by pouring from a narrow graduated cylinder. With closed pitchers of the Sarracenias, with plugged pitchers of this genus, and with all three types of pitchers of *Darlingtonia californica*, the top of the pitcher was cut off just beneath the lips or pitcher rim; the milk was introduced; the uppermost portion of the pitcher was folded over; a piece of paper bearing a memorandum of the experiment was included in the fold; one or more pins were passed through the doubled pitcher and paper, effectually sealing the pitcher cavity. With open pitchers of the Sarracenias, the milk was introduced through the open mouth of the pitcher which was then sealed in the manner just described.

At the end of a given period of time, the volume of the liquid contents of the pitcher was determined in the manner already described. When more than one pitcher was used in an experiment, the average volume of liquid contents per pitcher was determined at the end of the period of stimulation. The "Percentage increase above normal liquid contents" was calculated as follows. From the average volume of liquid contents per pitcher at the end of the period of stimulation was subtracted the sum of the volume of milk introduced plus the average volume of the liquid contents per pitcher in pitchers of the same type and species, using the values given in Table I. The remainder, which represented the volume of liquid secreted by the pitcher in response to stimulation, was divided by the average volume of the liquid contents per pitcher in pitchers of the same type and species as recorded in Table I. The quotient was multiplied by 100. The final arithmetical result expressed the volume of the liquid produced by stimulation as percent of the volume of liquid usually present in pitchers of the type and species used in the experiment. The average values in Table I were used, since the determinations

reported in that table and the stimulation experiments were made at the same time and place for a given species.

TABLE III.—RESPONSE OF THE PITCHERS OF CERTAIN *SARRACENIACEÆ* TO STIMULATION BY MILK.

| *Genus and Species* | *Number of pitchers.* | *Type of pitcher.* | *Volume of milk introduced into each pitcher.* | *Period of stimulation. Days.* | *Percentage increase above normal liquid contents.* |
|---|---|---|---|---|---|
| *Darlingtonia californica* | 7 | closed | 1 | 1 | 20 |
| " " | 12 | " | 3 | 1 | 86 |
| " " | 8 | " | 3 | 1 | 38 |
| " " | 6 | " | 3 | 3 | 200 |
| " " | 4 | " | 3 | 6 | 401 |
| " " | 1 | " | 12 | 6 | 1242 |
| " " | 7 | plugged | 1 | 1 | 37 |
| " " | 6 | " | 1 | 3 | 63 |
| " " | 3 | " | 15 | 6 | 532 |
| " " | 1 | " | 5 | 7 | 476 |
| " " | 4 | " | 12 | 7 | 446 |
| " " | 6 | open | 3 | 3 | 57 |
| " " | 1 | " | 15 | 5 | 292 |
| " " | 2 | " | 30 | 5 | 430 |
| " " | 6 | " | 3 | 6 | 119 |
| " " | 1 | " | 15 | 6 | 328 |
| " " | 1 | " | 45 | 6 | 435 |
| " " | 1 | " | 10 | 10 | 471 |
| " " | 1 | " | 30 | 10 | 578 |
| *Sarracenia Sledgei* | 4 | plugged | 5 | 5 | 952 |
| " *flava* | 4 | closed | 10 | 4 | 1096 |
| " " | 3 | open | 10 | 4 | 268 |
| " *Drummondii* | 4 | plugged | 5 | 5 | 506 |

The results, which have been summarized in Table III, demonstrate that the pitchers of the species studied poured out additional pitcher liquor in response to their stimulation by the introduced milk. The volume of additional liquor thus secreted depended in part on the volume of milk introduced, and in part on the period of time during which the stimulus was permitted to act.

When 30 cc. or more of milk were introduced into a pitcher, it became necessary to support the pitcher.

In all the experiments on food stimulation, injury to the pitcher was observed in but one experiment, which has not been included in those tabulated. An open pitcher of *Darlingtonia californica* received 30 cc. of milk; 10 days later, the pitcher was characterized by a brown discoloration and shrunken areas indicative of serious injury. A similar condition has been noted in the field in pitchers of the Sarracenias which contained a very unusual bulk of insect captures.

*Beef broth.*—Raw lean beef was cut into fine strips, covered with water,

and permitted to simmer for one hour. The resulting broth was strained and cooled. The broth was used in stimulation experiments; the technic was exactly the same as when milk was used.

In all the experiments on *Darlingtonia californica*, the period of stimulation was 5 days. Each of 4 closed pitchers received 5 cc. of the broth; the stimulation produced an increase above the normal average volume of the liquid contents per pitcher of 387 percent. Each of 5 plugged pitchers received 10 cc. of broth; the resulting increase above the normal liquid contents was 308 percent. Fifteen cc. of the broth were introduced into an open pitcher; an increase above the normal liquid contents of 346 percent resulted.

In each of the experiments on *Sarracenia flava*, 9 pitchers were used; 10 cc. of broth were introduced into each pitcher; and the period of stimulation was 4 days. Closed pitchers showed an average increase above the normal liquid contents of 1787 percent, and a maximum increase of 2445 percent. Open pitchers showed an average increase of 683 percent, and a maximum increase of 1060 percent.

*Meat.*—Both raw beef and beef cooked by boiling were used in separate series of experiments on *Darlingtonia californica*. From 2 to 8 cubes (length of edge one-eight inch) of meat were dropped into each pitcher; the period of stimulation ranged between 5 and 7 days; details concerning the preparation of the pitchers and the measurement of the liquid contents have been given under the experiments with milk. An increase in the average volume of the pitcher contents was not noted when *cooked* beef was introduced into either plugged or open pitchers. The increase observed after the introduction of *raw* beef ranged from 48 percent in one lot of closed pitchers to 157 in one lot of open pitchers; intermediate values were noted in other pitchers of all three types.

The failure of the meat, raw or cooked, to produce a large increase in the volume of pitcher liquor, such as was produced by milk or by beef broth, is probably due to the fact that the solid meat exerted any stimulating action only on the small area of the pitcher lining with which it was in contact. The liquid foods occupied a larger volume and, therefore stimulated the far larger area of the pitcher lining with which they were in intimate contact. The bulk of the meat introduced did not equal that of the insect captures of many open pitchers; and an increase of the liquor to a volume far greater than that present in open pitchers was not to be expected.

*Other foods of animal origin.*—Experiments, similar to those with meat, were made on *Darlingtonia californica*, using raw egg white, cubes of the white of hard-boiled eggs, cheddar cheese, casein (Hammarsten), and fibrin. No definite response to the introduction of these substances was observed, probably for the same reasons as with meat.

*Inorganic salts.*—The following salts were used in aqueous solution: ferrous sulphate, ferric ammonium sulphate plus sodium acetate, potassium ferrocyanide, and potassium ferricyanide.

Five cc. of a 2 percent solution of ferrous sulphate were introduced into several pitchers of each of the following species: *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*. The pitchers were not injured at the end of several days, and an increase occurred in the volume of the pitcher liquor; in pitchers of *S. flava*, the liquor had increased to 4 times its normal volume.

A solution was prepared containing 2 grams of ferric ammonium sulphate (iron ammonium alum) and 2 grams of sodium acetate in each 100 cc.; the sodium acetate was added for its buffer effect, *i. e.*, to lessen the acidity otherwise imparted to the solution by the alum. This solution was introduced into pitchers of the three species mentioned above, 5 cc. to each pitcher. Several days later, the pitchers were obviously dying.

Potassium ferrocyanide and potassium ferricyanide were used in separate series of experiments on both closed and open pitchers of *Sarracenia flava*. Five cc. of a dilute solution of one of these salts were introduced into each pitcher; 4 days later the volume of the liquid contents of the pitcher was measured. Both salts produced an abundant secretion of pitcher liquor; this phenomenon was more marked in the case of potassium ferrocyanide. The increased secretion in one closed pitcher was almost 21 times the average normal secretion.

### Response to Introduced Acid and Alkali

In the experiments on stimulation with milk, the pitcher contents frequently became acid to litmus. In order to ascertain whether the observed increase in the volume of the pitcher secretion was due to milk *per se* or to the acidity developed, the following studies were made with dilute acids. The response to the acids led to similar experiments with dilute alkali. The technic was that used in the experiments with milk. Except in two experiments, the reagent was permitted to remain in the pitcher for 5 days; in the experiments with single pitchers of *Sarracenia Sledgei* reported in Table IV, the acid re-

mained in the pitchers for 8 days. The reagents used were hydrochloric acid (symbol HCl, either 0.05 or 0.2 percent aqueous solution), acetic acid (abbreviated AcOH, either 0.05 or 0.1 percent aqueous solution), and sodium hydroxide (symbol NaOH, either 0.02 or 0.05 percent aqueous solution).

The following conclusions are deduced from a study of the data reported in Table IV:

TABLE IV.—RESPONSE OF THE PITCHERS OF CERTAIN *SARRACENIACEÆ* TO INTRODUCED ACID AND ALKALI

| *Genus and species.* | *Number of pitchers.* | *Type of pitcher.* | *Reagent.* | *Volume of reagent introduced into each pitcher, cc.* | *Average volume of pitcher contents at end of experiment, cc.* | *Reaction of pitcher contents to litmus at end of experiment.* |
|---|---|---|---|---|---|---|
| *Darlingtonia californica* | 15 | Plugged | 0.2% HCl | 3.0 | 6.00 | Neutral |
| *Darlingtonia californica* | 6 | Open | 0.05% HCl | 3.0 | 5.40 | Neutral |
| *Darlingtonia californica* | 1 | " | 0.05% HCl | 10.0 | 11.00 | Neutral |
| *Darlingtonia californica* | 7 | " | 0.2% HCl | 3.0 | 5.65 | Neutral |
| *Darlingtonia californica* | 6 | " | 0.2% HCl | 10.0 | * | Acid |
| *Darlingtonia californica* | 2 | " | 0.2% HCl | 30.0 | * | Acid |
| *Sarracenia Sledgei* | 2 | " | 0.05% HCl | 5.0 | 2.20 | † |
| " " | 1 | " | 0.05% HCl | 5.0 | <0.50 | Neutral |
| " *flava* | 1 | Closed | 0.05% HCl | 5.0 | 3.60 | Faintly acid |
| " " | 2 | Open | 0.05% HCl | 5.0 | 5.00 | Faintly acid |
| " *Drummondii* | 5 | " | 0.05% HCl | 5.0 | 3.55 | Neutral |
| *Darlingtonia californica* | 17 | Plugged | 0.1% AcOH | 3.0 | 6.15 | Neutral |
| *Darlingtonia californica* | 1 | Open | 0.1% AcOH | 3.0 | 6.00 | Neutral |
| *Sarracenia Sledgei* | 2 | " | 0.05% AcOH | 5.0 | 2.10 | Neutral |
| " " | 1 | " | 0.05% AcOH | 5.0 | <0.50 | Neutral |
| " *flava* | 1 | Closed | 0.05% AcOH | 5.0 | 2.80 | Neutral |
| " " | 3 | Open | 0.05% AcOH | 5.0 | 2.67 | Acid |
| " *Drummondii* | 4 | " | 0.05% AcOH | 5.0 | 1.20 | Neutral |
| *Darlingtonia californica* | 15 | Plugged | 0.02% NaOH | 3.0 | 6.07 | Neutral |
| *Darlingtonia californica* | 4 | Open | 0.02% NaOH | 3.0 | 6.80 | Neutral |
| *Sarracenia Sledgei* | 2 | " | 0.05% NaOH | 5.0 | 4.50 | Neutral |
| " *flava* | 1 | Closed | 0.05% NaOH | 5.0 | 4.00 | Neutral |
| " " | 1 | Open | 0.05% NaOH | 5.0 | 2.40 | Neutral |
| " *Drummondii* | 3 | " | 0.05% NaOH | 5.0 | 3.60 | Neutral |

*Darlingtonia californica* showed no marked trend to either increase or decrease the volume of its pitcher contents as a result of the introduction of dilute acid or dilute alkali.

* Pitchers injured, large areas brown and shrivelled.

† Contents of one pitcher faintly acid, of other pitcher neutral.

Almost invariably the volume of the liquid withdrawn from the pitchers of the Sarracenias was less than the volume of the introduced reagent. This shrinkage in volume was even greater than that shown by a comparison of the volume of reagent introduced with the average volume of the pitcher contents at end of experiment as given in Table IV, for the normal volume of pitcher liquor, which has already been reported in Table I, has not been included in Table IV. Therefore, evidence of absorption in the genus *Sarracenia* is unmistakable.

The reaction of the withdrawn pitcher contents was determined by means of litmus paper. The reaction, recorded in Table IV, should be compared with the reaction of the liquor of similar pitchers of the same species as reported in Table II. The pitcher contents of *Darlingtonia californica* returned to their normal neutral reaction within 5 days after the introduction of either dilute acid or dilute alkali. The contents of open pitchers of both *Sarracenia Sledgei* and *S. Drummondii* finally returned to their normal neutral reaction after introduction of any one of the three reagents. *Sarracenia flava* likewise showed a marked tendency for a return of its pitcher contents to their normal reaction within a few days after the introduction of either dilute acid or dilute alkali. This return of the pitcher contents to their normal reaction recalls the behavior of the human stomach under somewhat similar conditions as shown by the researches of Spencer, Meyer, Rehfuss, and Hawk.[70]

## Action of the Pitcher Liquor of the Sarraceniaceæ upon Living Insects

Even prior to the opening of the pitcher, the pitcher liquor of certain species possesses the property of quickly rendering helpless those insects which come into contact with it. Reference has already been made to the work of Mellichamp[4,16] and Watson,[17] who studied the action of the pitcher liquor of *Sarracenia variolaris* (*S. minor*) upon insects. In the present research similar studies have been made, using the pitcher liquor of four other species of the *Sarraceniaceæ*. Unless otherwise stated, the pitcher liquor was collected on the same day that its action on insects was tested.

A typical experiment may be described. A location was chosen adjacent to a populous crater-shaped nest of a large brown ant abundant at Summerville, South Carolina. Wide mouth vials were used as containers. Pitcher liquor from *Sarracenia flava* was introduced into 10 vials, 2 cc. to the vial; 5

vials received liquor from closed pitchers and 5 vials liquor from open pitchers. Water was placed in another set of 10 vials, 2 cc. to the vial, in order to serve as a control experiment. The vials were of uniform size, and were arranged in suitable order for convenient observation. Worker ants were picked from the nest gently with the fingers; one ant was dropped into each vial, and the time recorded. Note was made of the time at which each ant ceased its struggles to escape, assumed a rigid position, and became incapable of motion even on jarring the vial. The elapsed time between the introduction of the ants and the cessation of their struggles ranged from 0.5 to 3.0 minutes with an average of 1.4 minutes in the experiments with liquor from closed pitchers, and from 1.5 to 2.0 minutes with an average of 1.6 minutes in the experiments with liquor from open pitchers. In the control experiments with water, 5 of the 10 ants ceased motion, the elapsed time ranging from 3.0 to 9.5 minutes, while the other 5 ants were still active at the end of 30 minutes.

Liquor from open pitchers of *Sarracenia flava* was collected at De Funiak Springs, Florida, in May. It was kept at room temperature without addition of a preservative, and was tested at Wilmington, Delaware, 49 days later, using the above technic. Large black ants of the genus *Campanotus* served as the experimental animals, 6 ants in the experiment proper and 6 ants in the control experiment. The average elapsed time to cessation of motion was 20 *seconds* in the experiment proper. In the control experiment, 3 of the ants ceased motion in from 5.25 to 7.45 *minutes*, while the other 3 ants were still active at the end of 46 *minutes*.

Experiments were made with the pitcher liquor of *Sarracenia Sledgei* at Mobile, Alabama, using large black ants of the genus *Campanotus*. The average elapsed time to cessation of motion was 48 seconds when 5 ants were introduced into liquor from open pitchers. Five ants were used in the control experiments, 1 ceased motion at the end of 180 seconds, the others were still active at the end of 300 seconds.

Experiments were also made on liquor from open pitchers of *Sarracenia Drummondii* at De Funiak Springs, Florida, using large brown ants. Five ants were introduced into the pitcher liquor and ceased motion in from 20 to 30 seconds; 5 ants which were introduced into distilled water, retained their motility for many minutes, and were finally permitted to escape at the conclusion of the experiment.

Typical experiments with the pitcher liquor from *Sarracenia Sledgei*,

*S. flava*, and *S. Drummondii* have been reported above. Similar results, concerning the action of the pitcher liquor on insects, have been obtained in other experiments—not reported in detail—on liquor from closed pitchers and from open pitchers of these species. The action of the pitcher liquor of *Darlingtonia californica* upon insects is described in a subsequent section.

Death of the ants was not coincident with their cessation of motion. Thus in the second experiment reported above (that made on black ants at Wilmington, Delaware), 6 ants ceased motion, on the average 20 seconds after their introduction into the pitcher liquor. Fifteen minutes after motion had ceased, they were removed from the pitcher liquor and placed on bibulous paper. They showed evidences of motion, on the average, 6 minutes, 22 seconds later, and were able to run away, on the average, 9 minutes after their removal from the liquor. The ants from other experiments behaved in a similar manner. However, permanent cessation of motion of the captured insects must inevitably lead to their death within the pitchers.

Insects sank in the pitcher liquor of the Sarracenias more frequently and more promptly than in water in the control experiments. This phenomenon suggested that the pitcher liquor has a surface tension less than that of water, and acts upon the insects by virtue of this property. Chemical, physical, and physiological tests were made in this connection.

### Chemical, Physical, and Physiological Examination of the Pitcher Liquor

These tests were made with pitcher liquor to which no bactericide had been added.

*Test for saponin.*—Since saponins lower the surface tension of water when dissolved in that solvent, the hemolysis test for saponins was applied to samples of the pitcher liquor in order to ascertain if compounds of that group were present. The finger was pricked and blood was permitted to fall into 0.5 cc. of a 0.1 percent solution of sodium citrate in physiological (0.85 percent) solution of sodium chloride, until the volume of the latter had doubled. Equal volumes of the resulting suspension of human erythrocytes and the pitcher liquor were mixed on a glass slide, covered with a cover slip, and then viewed through a microscope. The erythrocytes remained intact at the end of 30 minutes; therefore, hemolysis did not occur, and saponins were not present in the pitcher liquor. This test was applied to samples of liquor from closed pitchers of *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drum-*

*mondii*, and from open pitchers of *S. flava* and *S. purpurea*. With all 6 samples, the test for a saponin was invariably negative.

The *physical tests* were both qualitative and quantitative. An oiled needle was used for a qualitative measurement of the surface tension. A needle 1.25 inches in length was oiled with olive oil. It readily floated on the surface of water for its entire length, and was not readily sunken by jarring the sides of the containing vessel. The oiled needle was also placed on the surface of the liquor from open pitchers of *Sarracenia flava* which was used in the experiments made on ants at Wilmington, Delaware, and reported above. The needle was floated on the surface of the pitcher liquor with difficulty. The needle then floated with both ends submerged, and sank very readily when the sides of the containing vessel were gently jarred. The same oiled needle was transferred, by means of dissecting forceps, 20 times in succession from the water to the pitcher liquor, then back to the water. *Throughout the entire series of transfers*, the needle was readily floated on the water and was not sunken in that medium by a heavy jarring of the table; on the other hand, the needle was floated on the pitcher liquor with difficulty and readily sank in the liquor on the slightest jarring of the table.

Quantitative measurement of the surface tension of the liquor from closed pitchers of *Sarracenia flava* was made by means of the du Noüy surface tension apparatus,[71] as manufactured by the Central Scientific Company.[72] The measurements were made at a temperature of 25° C. The pitcher liquor was found to have a surface tension of 66.4 dynes per cm., while a sample of distilled water, tested at the same time, was found to have a surface tension of 75.0 dynes per cm. Hence the surface tension of the pitcher liquor was considerably lower than that of water.

*Physiological tests.*—The action of liquor from closed pitchers of *Sarracenia flava* was tested upon frogs and a guinea-pig.

The pitcher liquor was injected into the anterior lymph sac of *frogs;* 5 animals were used; the dose per gram of body weight ranged from 0.0035 to 0.05 cc., and the actual dose from 0.12 to 0.49 cc. The frogs were observed at intervals for 9 hours, and remained alive and active. The next day, two of the frogs were pithed. In both of them, the lymph sac showed no evidence of irritation; however, it was practically free from mucus which is normally present. Possibly the mucus had been digested by the proteolytic enzyme of the pitcher liquor.

One cc. of the pitcher liquor was injected subcutaneously into a *guinea-pig* weighing 527 grams; no evil effects were noted. Three days later, 3 cc. of the same sample of pitcher liquor were administered subcutaneously to the same guinea-pig without the production of any evil effects. Five days after the second injection the guinea-pig showed some adhesions in the region of the injections but was otherwise uninjured.

### Action of the Pitcher Liquor of *Darlingtonia californica* Upon Living Insects

The action of the liquor from both closed and open pitchers of *Darlingtonia californica* was tested on ants, locusts (short-horned grasshoppers, family *Acrididæ*), and bumble bees (genus *Bombus*). These experiments were made in Plumas County, California.

In one experiment, 25 vials were prepared, and 3 cc. of liquor from closed pitchers were introduced into each vial. For controls, 3 cc. of water were introduced into each of another group of 25 vials. A large ant was dropped into each vial. The number of ants still active and struggling to escape was:– At the end of 1 minute, pitcher liquor 18, water 17; at the end of 5 minutes, pitcher liquor 11, water 8; at the end of 10 minutes, pitcher liquor 8, water 8; at the end of 30 minutes, pitcher liquor, 6, water 8. These results showed practically no difference between the pitcher liquor and the water with respect to their action on ants.

Similar experiments were made with bumble bees and with locusts, using liquor from closed and from open pitchers separately; the period of observation extended over several hours in each experiment. The results showed that the action of the pitcher liquor on these insects with respect to motility did not differ materially from that of water.

None of the insects—ants, bees, or locusts—sank in the pitcher liquor more frequently or more promptly than in water.

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