emission from both planets is related to the gyrofrequency, a rather interesting relationship exists. The frequency at peak intensities is 0.3 Mhz for Earth and 8 Mhz for Jupiter (*13*), and the observed high-frequency cutoff is about 1.7 Mhz for Earth and 40 Mhz for Jupiter (*11*). The frequencies at peak intensity are thus in a ratio of 27 to 1, and the maximum frequencies are in a ratio of 24 to 1. If the magnetic field strengths of the two planets scale like the gyrofrequencies, the Jovian polar field derived by this method would be 15 to 18 gauss. The Pioneer 11 measurements (*14*) indicate a value of 14 to 23 gauss for the Jovian north pole. With Brown's (*2*) spectrum of the Saturn emissions shown in Fig. 3, we would, by the same logic, infer a polar surface field of 2 gauss for that planet. For a dipole field, this would correspond to 1 gauss at the equator, which is the field strength estimated by Scarf (*15*), using various lines of evidence. This prediction will undergo a crucial test in 1979 with the Pioneer 11 flyby of Saturn.

These similarities are circumstantial and speculative at this point, but we feel, nevertheless, that there may well be a pattern in these radio emissions. The Mariner Jupiter-Saturn missions to be launched in 1977 will carry sophisticated radio astronomy experiments as well as magnetic field and particle experiments capable of determining many of the parameters at Jupiter and Saturn. Thus, in the next 5 or 6 years the reality of our proposed pattern will become known.

M. L. KAISER
R. G. STONE
Radio Astronomy Branch, Laboratory for Extraterrestrial Physics, Goddard Space Flight Center, Greenbelt, Maryland 20771

References

1. J. W. Warwick, *Radio Astronomical and Satellite Studies of the Atmosphere*, J. Aarons, Ed. (North-Holland, Amsterdam, 1963), pp. 400–424.
2. L. W. Brown, *Astrophys. J.* **198**, L89 (1975).
3. E. A. Benediktov, G. G. Getmantsev, N. A. Mityakov, V. O. Rapoport, A. F. Tarasov, *Kosm. Issled.* **6**, 946 (1968).
4. N. Dunckel, B. Ficklin, L. Rorden, R. A. Helliwell, *J. Geophys. Res.* **75**, 1854 (1970).
5. R. G. Stone, *Space Sci. Rev.* **14**, 534 (1973).
6. D. A. Gurnett, *J. Geophys. Res.* **79**, 4227 (1974).
7. L. W. Brown, *Astrophys. J.* **180**, 359 (1973).
8. J. W. Warwick, *Particles and Fields Near Jupiter* (NASA Publication CR-1685, Government Printing Office, Washington, D.C., 1970).
9. J. Fainberg, L. G. Evans, R. G. Stone, *Science* **178**, 743 (1972).
10. T. R. Hartz, in *The Radiating Atmosphere*, B. M. McCormac, Ed. (Reidel, Dordrecht, Netherlands, 1971), pp. 225–238.
11. For a thorough review, see T. D. Carr and S. Gulkis, *Annu. Rev. Astron. Astrophys.* **7**, 577 (1969).
12. G. M. Gruber and C. Way-Jones, *Nat. Phys. Sci.* **237**, 137 (1972).
13. M. D. Desch and T. D. Carr, *Astrophys. J.* **194**, L57 (1974).
14. M. H. Acuña and N. F. Ness, *J. Geophys. Res.*, in press; E. J. Smith, L. Davis, Jr., D. E. Jones, P. J. Coleman, Jr., D. S. Colburn, P. Dyal, C. P. Sonett, *Science* **188**, 451 (1975).
15. F. L. Scarf, *Cosmic Electrodyn.* **3**, 437 (1973).

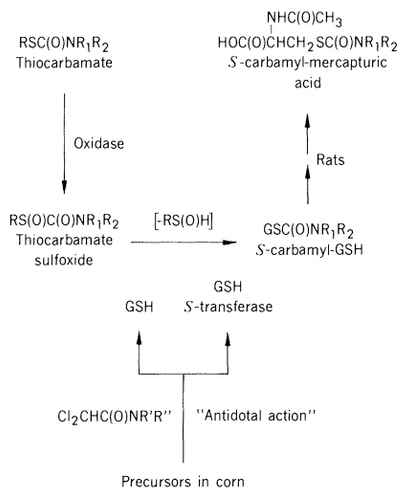
30 January 1975; revised 7 April 1975

Dichloroacetamide Antidotes for Thiocarbamate Herbicides: Mode of Action

Abstract. *Thiocarbamate sulfoxides formed on metabolic sulfoxidation of thiocarbamate herbicides in plants and mammals are effective carbamoylating agents for glutathione and other tissue thiols. Dichloroacetamides that protect corn from thiocarbamate herbicide injury induce more rapid detoxification of the thiocarbamate sulfoxides by increasing their rate of carbamoylation of glutathione through elevation of the root glutathione level and glutathione* S*-transferase activity.*

Two recent advances provide new chemical probes, thiocarbamate sulfoxides and dichloroacetamides, useful in elucidating the mode of action of thiocarbamates, one of the most important classes of herbicide chemicals. Biological oxidation to form thiocarbamate sulfoxides probably constitutes the first step in a chain of events leading to inhibition of plant growth (*1*). In thiocarbamate-susceptible corn varieties, this chain of events is apparently disrupted by dichloroacetamide "antidotes," which are effective adjuvants in preventing injury due to thiocarbamates (*2*). Three biochemical observations are also relevant. The metabolism of fatty acids is altered by EPTC (*S*-ethyl *N,N*-dipropylthiocarbamate) in some plant species (*3*), suggesting an interference with coenzyme A (CoASH)–mediated reactions. Thiocarbamate sulfoxides are cleaved by glutathione (GSH) *S*-transferase enzymes of mouse liver (*1*). The much greater tolerance of corn than of oat seedlings to thiocarbamates and their sulfoxides (*1*) extends to atrazine herbicide, a chemical which is metabolized by a GSH *S*-transferase of corn but not oat seedlings (*4*).

We now establish that thiocarbamate sulfoxides in vitro readily carbamoylate CoASH and GSH, important enzyme cofactors, and that the antidotes act in corn to elevate the GSH and GSH *S*-transferase levels, resulting in rapid detoxification of the thiocarbamate sulfoxides. The following scheme illustrates a portion of these reactions and relationships.



The studies were made with two thiocarbamates (EPTC and butylate), their sulfoxide derivatives prepared by peracid oxidation (*1*), and two dichloroacetamides (R-25788 and R-29148). These two thiocarbamates are used commercially in combination with the antidote R-25788: Eradicane®, which is EPTC plus antidote, and Sutan +®, which is butylate plus antidote (*5*).

EPTC	$CH_3CH_2SC(O)N(CH_2CH_2CH_3)_2$
Butylate	$CH_3CH_2SC(O)N[CH_2CH(CH_3)_2]_2$
R-25788	$Cl_2CHC(O)N(CH_2CH{=}CH_2)_2$
R-29148	$Cl_2CHC(O)N{-}CH_2{-}(CH_3)_2COCHCH_3$

Several initial observations served to focus attention on tissue thiols. The thiocarbamates are converted in mammals and plants to the corresponding sulfoxides, which do not accumulate since they are further metabolized. Thus, EPTC sulfoxide appears as a transient metabolite in the liver of mice 10 minutes after intraperitoneal administration of EPTC at 1.0 mmole/kg (*1*). Further, EPTC sulfoxide is detected in extracts of roots from oat seedlings exposed for 24 hours to [^{14}C]EPTC solutions and of leaves from corn seedlings 24 hours after injection of [^{14}C]EPTC into the stem. The transient nature of the thiocarbamate sulfoxides suggests that they react with tissue constituents such as thiols. This was verified by the finding that CoASH, GSH, and *N*-acetylcysteine are converted to *S*-carbamyl derivatives on reaction with equimolar amounts of either EPTC sulfoxide or butylate sulfoxide in an aqueous medium at *p*H 7.4 (*6*). The alkylsulfenic acids released from the thiocarbamate sulfoxides on carbamoylation of thiols are quite unstable in aqueous medium at physiological *p*H in the presence or absence of biological material, giving predominately the corresponding alkylsulfonic acids (*7*). The thiocarbamate sulfones are even more effective carbamoylating agents than the corresponding sulfoxides, but this observation is probably not relevant to the mode of action of thiocarbamate herbicides since the sulfones are much less effective as herbicides (*1*) and they are not detected as in vivo metabolites of the thiocarbamates in mammals (*1*) or plants.

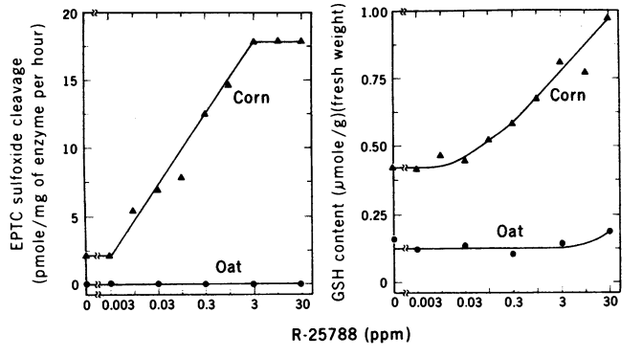


Fig. 1. Effect of exposure for 24 hours of corn and oat seedlings to various concentrations of R-25788 on the root glutathione *S*-transferase or on EPTC sulfoxide cleavage activity, and on the root glutathione content.

The complete inactivity of the thiocarbamates themselves as carbamoylating agents under physiological conditions leads to the hypothesis that the thiocarbamates are oxidized to the corresponding sulfoxides, which then serve as carbamoylating agents for important tissue thiols, such as CoASH and GSH, with or without enzymatic mediation. Whereas the relevance to reactions in vivo of the carbamoylation of CoASH or of other components of CoASH-mediated systems remains to be evaluated, the importance of GSH carbamoylation is firmly established by observations in both mammals and plants, as discussed below.

Male mice and rats were used to evaluate the importance of GSH in mediating the fate in mammals of thiocarbamate herbicide chemicals. The concentration of GSH in the liver (*8*) 3 hours after intraperitoneal administration of EPTC or EPTC sulfoxide (1.5 mmole/kg) to mice is reduced by 26 and 49 percent, respectively, relative to comparable control animals. The reduced GSH content probably results from carbamoylation of the GSH, mediated by GSH *S*-transferase enzymes (*1*), either by the administered sulfoxide or that formed on in vivo sulfoxidation of the thiocarbamate. Conjugates of GSH are normally cleaved and acetylated to form the corresponding mercapturic acids prior to excretion by mammals (*9*). Analysis of the urine (*10*) collected within 24 hours after administration of either EPTC, EPTC sulfoxide, or butylate (0.6 to 1.0 mmole/kg) to rats revealed the corresponding *S*-(*N*,*N*-dialkylcarbamyl)-mercapturic acids in the indicated amounts relative to the administered thiocarbamate or thiocarbamate sulfoxide dose: 5 to 8 percent after oral or intraperitoneal administration of EPTC; 12 percent from intraperitoneal administration of EPTC sulfoxide; 1 percent after oral or intraperitoneal administration of butylate. It appears, therefore, that one pathway for thiocarbamate herbicide metabolism in mammals involves sulfoxidation, carbamoylation of GSH, and degradation of the GSH conjugate to form the corresponding mercapturic acid.

One of several mechanisms by which an additive or antidote might influence the potency of a herbicide involves an alteration in the rate of herbicide detoxification. Accordingly, the thiocarbamate sulfoxide, GSH, GSH *S*-transferase system was examined in the roots of corn and oat seedlings exposed to dichloroacetamides at various concentrations. Germinated corn seedlings (DeKalb XL-66H) with an average root length of 3 to 4 cm and oat seedlings (Curt) with an average root length of 2 to 3 cm were placed on filter paper in petri dishes containing the test solutions so that the roots were partially immersed in the aqueous medium. After 24 hours exposure to selected concentrations of the dichloroacetamides, the roots were cut off, washed with distilled water, and analyzed for GSH content (*8*) and for activity of the root homogenates fortified with GSH in cleavage of EPTC sulfoxide and butylate sulfoxide (*11*).

Dichloroacetamides R-25788 and R-29148 have two concentration-dependent effects on corn seedlings that may be important in reducing corn injury from thiocarbamates. As illustrated in Fig. 1 with R-25788 and EPTC sulfoxide, this antidote elevates the GSH level twofold and the GSH *S*-transferase activity ninefold (*12*). The threshold level of antidote for increased GSH *S*-transferase activity is extremely low, below 0.01 part per minute (ppm) or $5 \times 10^{-8} M$, and a plateau is reached at 3 ppm; beyond this limit no further stimulation occurs. Similar relations are found with the R-29148–EPTC sulfoxide and R-25788–butylate sulfoxide combinations. The activity of the enzyme system in metabolizing the sulfoxides is dependent on GSH fortification. The responsible enzyme is likely to be a GSH *S*-transferase since the principal product of the enzyme reaction with butylate sulfoxide is identified as *S*-(*N*,*N*-diisobutylcarbamyl)-GSH (*13*) and this product is formed in greater amount with enzyme from plants treated with R-25788 than from plants that were not treated. Thus, the antidotes acting in corn increase both the GSH *S*-transferase and GSH, its essential cofactor, which probably results in increased detoxification of the sulfoxides by carbamoylation of the GSH. Oat seedlings also respond to R-25788 with increased GSH concentrations, particularly with antidote concentrations above the 30 ppm shown in Fig. 1, but their activity for metabolizing EPTC sulfoxide is not elevated. However, their level of detoxifying enzyme and cofactor is very low, relative to corn, in any case. Since EPTC sulfoxide is eight times more potent than EPTC in inhibiting oat root elongation (*1*) and antidote R-25788 is not active with oats with either EPTC (*2*) or its sulfoxide, it appears that the level of GSH *S*-transferase activity contributes to the species specificity noted between oat and corn seedlings.

The new chemical probes, thiocarbamate sulfoxides and dichloroacetamide antidotes, used here in investigating thiocarbamate herbicide mode of action may provide a new and useful variable in studies on GSH synthesis and functions. They may also prove useful as general biochemical reagents acting in a manner similar to that in which they serve so importantly in the control of noxious weeds.

MING-MUH LAY, JAMES P. HUBBELL
JOHN E. CASIDA
Division of Entomology and Parasitology, University of California, Berkeley 94720

References and Notes

1. J. E. Casida, R. A. Gray, H. Tilles, *Science* **184**, 573 (1974); J. E. Casida, E. C. Kimmel, M.-M. Lay, H. Ohkawa, J. E. Rodebush, R. A. Gray, C. K. Tseng, H. Tilles, *Environ. Quality Safety*, in p → J. E. Casida, E. C. Kimmel, H. Ohkawa, R. Ohkawa, *Pest. Biochem. Physiol.* **5**, 1 (1975).
2. F. Y. Chang, J. D. Bandeen, G. R. Stephenson, *Can. J. Plant Sci.* **52**, 707 (19 → *Weed Res.* **13**, 399 (19 → F. Y. Chang, G. R. Stephenson, J. D. Bandeen, *Weed Sci.* **21**, 292 (19 → *J. Agr. Food Chem.* **22**, 245 (1974); F. M. Pallos, M. E. Brokke, R. A. Gray, D. R. Arneklev, paper presented at the meeting of the Division of Pesticide Chemistry, American Chemical Society, Chicago, Ill., August 1973; Stauffer Chemical Co., Belgian Patent 782,120 (1972); Belgian Patent 806,038 (1974).
→ W. A. Gentner, *Weeds* **14**, 27 (19 → J. L. Harwood and P. K. Stumpf, *Arch. Biochem. Biophys.* **142**, 281 (1971); P. E. Kolattukudy and L. Brown, *Plant Physiol.* **53**, 903 (1974); G. G. Still, D. G. Davis, G. L. Zander, *ibid.* **46**, 307 (1970); R. E. Wilkinson, *ibid.* **53**, 269 (19 → _____ and W. S. Hardcastle, *Weed Sci.* **18**, 125 (1970); _____, A. E. Smith, paper presented at the meeting of the Weed Science Society of America, Las Vegas, Nev., February 1974.
→ D. S. Frear and H. R. Swanson, *Phytochemistry* **9**, 2123 (1970); _____, F. S. Tanaka, *Recent Adv. Phytochem.* **5**, 225 (1972).
5. *Herbicide Handbook* (Weed Science Society of America, Champaign, Ill., ed. 3, 1974).
6. *S*-(*N*,*N*-Dialkylcarbamyl) derivatives isolated by thin-layer chromatography (TLC) and fractional crystallization (GSH and mercapturic acid derivatives only) were identified by nuclear magnetic resonance (NMR) spectroscopy and functional group tests with appropriate chromogenic agents.
7. Alkylsulfonic acids were identified by NMR and infrared spectroscopy.
8. GSH was determined by homogenizing the samples of liver and plant roots in 5 percent trichloroacetic acid, removing the protein precipitate by centrifugation, neutralizing the supernatant with NaOH, and then reacting the supernatant with 5,5′-dithiobis(2-nitrobenzoic acid) [P. C. Jocelyn, *Biochemistry of the SH Group* (Academic Press, New York, 19 → M. Koivusalo and L. Uotila, *Anal. Biochem.* **59**, 34 (1974)].
→ L. F. Chasseaud, *Drug Metab. Rev.* **2**, 185 (1973); J. L. Wood, in *Metabolic Conjugation and Meta-*

bolic Hydrolysis, W. H. Fishman, Ed. (Academic Press, New York, 1970), vol. 2, p. 261.
10. The methanol-soluble portion of the residue from urine after extraction with ether and lyophilization to dryness was treated with diazomethane and then subjected to gas-liquid chromatography (GLC) analysis on a 3.8 percent OV-101 or 3.0 percent OV-17 column at 150° to 250°C with quantitation of the methylated mercapturic acids using a flame ionization detector. The structural identity of each excreted mercapturic acid was verified by methylation and GLC-mass spectroscopy (chemical ionization) comparison with authentic standards.
11. The standard plant root detoxification assay involved addition of [*S*-alkyl-^{14}C]EPTC sulfoxide (1 nmole) or [*N*-alkyl-^{14}C]butylate sulfoxide (0.5 nmole) to the 17,300*g* supernatant of root homogenates (2.5 to 320 mg of fresh tissue weight equivalent) and GSH (10 μmole) in phosphate buffer (0.1*M*, *p*H 6.8, 1.1 ml). After 2 hours incubation at 25°C, the unmetabolized [^{14}C]EPTC sulfoxide or [^{14}C]butylate sulfoxide was recovered by extraction with chloroform and subjected to liquid scintillation assay. Enzyme activity is expressed as picomoles of thiocarbamate sulfoxide cleaved per milligram of enzyme (fresh tissue weight equivalent) per hour.
12. In a separate but similar study in which were assayed the GSH *S*-transferase activity with EPTC sulfoxide at a high substrate level (1 μmole), corn root enzyme (5 to 40 mg of fresh tissue weight equivalent) prepared with insoluble polyvinylpyrrolidone (*4*) and 40 minutes of incubation, the same relationship was noted of increasing enzyme activity with increasing R-25788 level. In this case, the enzyme activity was elevated two- to fourfold at high antidote levels as compared with no antidote treatment.
13. *S*-(*N*,*N*-[^{14}C]Diisobutylcarbamyl)-GSH formed as a metabolite of [^{14}C]butylate sulfoxide (*11*) was identified by cochromatography with an authentic unlabeled standard (*6*) on two-dimensional TLC with 0.5-mm silica gel F_{254} chromatoplates developed in the first direction with pyridine, isopropanol, water, glacial acetic acid system (100 : 20 : 20 : 1) and in the second direction with the same system in the ratio of 50 : 55 : 45 : 2.
14. Supported in part by PHS grant PO1 ES00049 and PHS fellowship 1 F22 ES00681 (the latter to J.P.H.). R. L. Holmstead of this laboratory assisted in the GLC—mass spectroscopy studies. We thank R. A. Gray and F. M. Pallos of Stauffer Chemical Co. for samples of [^{14}C]EPTC, [^{14}C]-butylate, R-25788, and R-29148. The corn and oat seeds were provided by C. W. Crum of Dekalb Agricultural Research, Inc., DeKalb, Ill., and C. O. Qualset of the University of California, Davis, respectively.

5 January 1975; revised 25 February 1975

Nectar: Its Production and Functions in Trumpet Creeper

Abstract. *Studies of the trumpet creeper,* Campsis radicans *(L.) Seem. (Bignoniaceae), reveal five distinct nectary systems, a phenomenon never before reported among temperate zone plants. Ant activity, centered around the four extrafloral systems, clearly demonstrates the ant-guard symbiosis usually associated only with tropical or subtropical species. Floral nectar, an attractant for hummingbird and bumblebee pollinators, differs chemically from the ant-attracting nectar produced extraflorally.*

The production of nectar by plants and the attraction of certain insects to nectar have been of long-standing fascination to scientists (*1*). Much work has been channeled into the study of floral nectaries and the pollinators associated with them (*2*). Investigations of extrafloral nectaries have been limited until recently. These structures are located on the outer floral (*3*) and vegetative parts, including petioles (*4*), sheaths (*5*), and leaf margins (*6*). A result of this recent work has been a broadening of the field of animal-plant interactions. In recent years many ant-plant associations have been recognized in the tropics and subtropics, ranging from casual temporary alliances to mutualistic symbioses in which the participants are dependent on each other for survival. An example of the latter is the bull's horn acacia, *Acacia cornigera* L., and the acacia ant, *Pseudomyrmex ferruginea* F. Smith (*7*). The ants inhabit the enlarged hollowed stipular thorns and feed on a balanced diet of nectar from petiolar nectaries and Beltian bodies, modified leaf tips rich in protein. In addition to removing encroaching plants, the resident ants drive off invading insects by biting and stinging.

The trumpet creeper, *Campsis radicans*, one of the few temperate representatives of the tropical and subtropical family Bignoniaceae, is a common woody vine of the eastern and midwestern United States. Throughout its range it is host to several genera of ants. The relationship is not obligatory, although varying degrees of protection may be offered by different ant species in return for the extrafloral nectar produced by the plant. Trumpet creeper is possibly unique among temperate species, and certainly among a small number of all nectariferous plants, in possessing five nectary systems. The four extrafloral systems, located on the petiole (Fig. 1, a, b, and d), calyx (Fig. 1, e and f), corolla, and fruit (Fig. 1, h and i), are each visited regularly by ants. This report is perhaps the first documented case of nectaries occurring on developing fruits of any species. The ovarian (floral) nectary (Fig. 1g) aids in attracting the primary and secondary pollinators, hummingbirds and bumblebees, respectively. The extrafloral systems are also present on the Old World *Campsis grandiflora* as well as its hybrid *Campsis* × *tagliabuana* (*C. radicans* × *C. grandiflora*).

The aggressive climbing habit of the trumpet creeper has made it a familiar sight along hedgerows and fences of the Midwest. The flowers are five-parted and bilaterally symmetrical, each with a short tubular calyx and a flaring, tubular, showy, bright orange corolla, borne in dense terminal corymbs of 12 to 35 flowers (n = 21.6) (Fig. 1, c and j). Leaves are opposite, pinnately compound, each with 7 to 11 leaflets. The hybrid closely resembles its American parent in gross morphological features, the most conspicuous differences being the looser inflorescences and the larger flowers. Flowering, which occurs in both taxa from early July until early September, follows the "cornucopia" pattern as described by Gentry (*8*). Fruiting continues into late September, averaging 2.6 fruits per inflorescence.

Material was studied during the summers of 1972 and 1974 from native populations in southern Illinois, and naturalized populations were examined in southeastern New York. The hybrid was grown and observed in the nursery of the Cary Arboretum. Living material was fixed and stored in FAA (formalin, acetic acid, and ethyl alcohol), dehydrated in a tertiary butyl alcohol series, and embedded in TissuePrep. Serial sections were made at 10 μm, stained with safranin, and counterstained with fast green or with Delafield's hematoxylin. In order to determine the carbohydrate components, we collected nectar at different times during the day throughout the growing season, using microcapillary spotting tubes. Analysis was accomplished by thin-layer chromatography (*9*).

The individual extrafloral nectaries are minute and may be easily overlooked by the casual observer. The nectaries are generally circular in outline with a well-defined structure of a cup cavity with a base, surrounded by a wall or rim. Data concerning location, number, and size of the different nectaries and nectar composition are given in Table 1.

The petiolar nectaries are the first to secrete. The nectaries on the youngest three or four pairs of petioles on each branch

Table 1. Nectary systems of the trumpet creeper. Abbreviations: S, sucrose; G, glucose; F, fructose.

Location	Average number	Average height (mm)	Average diameter (mm)	Ratio of sugars		
				S	G	F
Extrafloral						
Petiole—adaxial surface	15.6/petiole	0.14	0.27	1	1	1
Calyx—abaxial lobes	20/flower	0.17	0.26	3	2	1
Corolla—abaxial lobes	25/flower	0.14	0.23	3	2	1
Fruit—scattered	200/fruit	0.15	0.19	1	1	1
Floral						
Ovarian—base of style	1/flower	1.73	3.98	0	1	1