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Triazolines. XXIX. 1,5-Diaryl-Δ^2-1,2,3-triazolines as Aphicides: Mechanism of Action via Aziridine Formation

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Abstract: The aphicidal activity of 21 different 1,5-diphenyl-Δ^2-1,2,3-triazolines, conveniently prepared utilizing the catalytic effect of water on the 1,3-cycloaddition of diazomethane to Schiff bases in aqueous dioxane, was evaluated. Triazolines bearing an *o*-Cl substituent on the C-phenyl, either alone (**4**) or in combination with a *m*- and/or a *p*-substituent on the *N*-phenyl (**14**, **15**, **17** and **18**), showed significant activity, with a combined *m*-, *p*- substitution on the *N*-phenyl the most effective (**17** and **18**). While an *o*-Cl substituent led to greater activity than an *o*-NO_2 group, the introduction of an additional *p*-Cl substituent on the C-phenyl eliminated activity (**21**).

The aphicidal activity of triazoline **18** was found to be dependent on the presence of UV light. Since fluorescent lighting used in the testing procedure contains UV light and since triazolines undergo photolysis when exposed to UV light to yield aziridines, it was logical to conclude that the aphicidal activity of the triazolines was, in fact, derived from the aziridines formed during the testing procedure. This mechanism of action was confirmed by preparing the aziridines **22**, **23** and **24** corresponding to the active triazolines **14**, **15** and **18**, and showing that they possessed aphicidal activity equal to or better than that of the triazolines, and by the activity observed in several other structurally related aziridine analogues (**25**–**28**). Unlike aziridinyl phosphorous compounds, the aziridines described here are not mutagenic in the Ames assay and thus afford a safer class of pesticides.

1 INTRODUCTION

Studies in the author's laboratories on the role of protic and dipolar aprotic solvents in 1,3-cycloaddition reactions have led to a versatile method of general utility[1–5] for the synthesis of Δ^2-1,2,3-triazolines.[6–8] The procedure utilizes the accelerating effect of protic solvents such as water on the 1,3-cycloaddition of diazomethane to Schiff bases (arylidene anilines) for triazoline synthesis, and a variety of previously unknown 1,5-diaryl-Δ^2-1,2,3-triazolines are now readily available in sufficient quantities to permit a detailed screening of these compounds for biological activity. Since effective methods of synthesis were previously lacking,[9,10] the biological properties of this novel group of heterocycles have not been well explored. Existing literature consists only of previous reports from our own laboratories on the results of screening a number of 1,5-diaryltriazolines for herbicidal[11] and pesticidal[12] activity. Several 1,5-diaryl- and 1-aryl-5-heteroaryltriazolines have also been screened for anticonvulsant properties.[7,8,13,14] This paper describes findings on the aphicidal activity of the triazolines and

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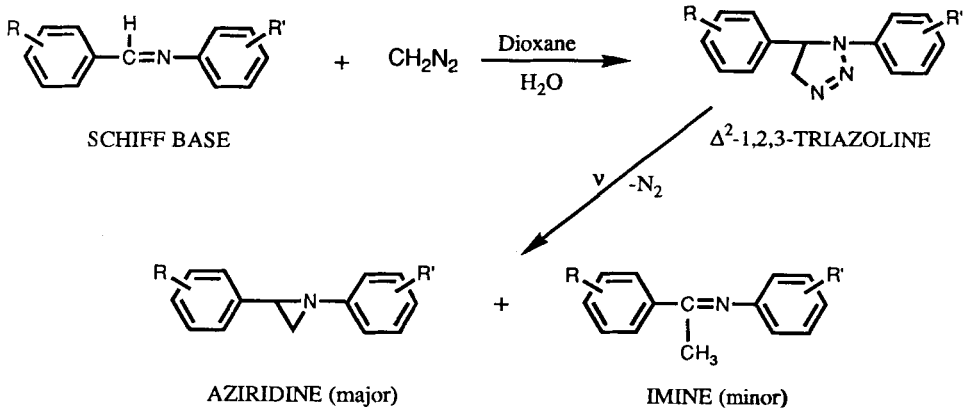

Fig. 1. Summary of synthesis of triazolines and aziridines.

proposes that aziridine formation is responsible for the observed biological activity.

2 EXPERIMENTAL METHODS

2.1 Screening procedures

The testing procedure consisted of a spray test using nasturtium plants (*Tropaeolum majus* L., var. Tall Single) and bean aphids, *Aphis fabae* (Scopoli). The test chemical (the triazolines **1–21** or the aziridines **22–29**) was dissolved in acetone; 50 ml of this solution was emulsified with 'Triton' X-155 in water to give a test formulation containing 1 g a.i. liter^{-1}. Lower concentrations were obtained by standard serial dilution techniques.

The nasturtiums were infested with aphids (100–200 aphids per plant) and were then sprayed with the triazoline test formulations and placed under a fluorescent lamp in a tray and given bottom watering for the duration of the test. Dead aphids were collected and counted in a cup device that was attached to the set-up. Percentage mortality was determined after a three-day period.

Aphicidal activity of triazoline **18**, in the presence and absence of UV light, was determined as follows: the tests were repeated in the manner described above, except that the fluorescent light was filtered through UV-absorbing Mylar W_2 sheets. The effect of UV light on the test compound was evaluated by irradiating an acetone solution of **18** with UV light in the 354 nm region, the λ_{max} for most triazolines.[6] The lamp was held at a distance of 4 in for 2 h and the resulting solution was tested for aphicidal activity in the absence of fluorescent light.

2.2 Synthesis of 1,5-diaryl-1,2,3-triazolines and 1,2-diarylaziridines

The triazolines **1–21** were synthesized following the convenient procedure developed in the author's laboratories (Fig. 1).[1–5] In a typical experiment, the Schiff base (anil) was dissolved with cooling if necessary in a freshly-prepared solution of diazomethane in aqueous dioxane. The reaction mixture was then allowed to stand at room temperature for two to four days for the reactive anils, and six to seven days for the less active ones. At the end of this period, the mixture was cooled and diluted with cold water until a permanent precipitation appeared. Further cooling yielded the triazoline as a clean crystalline material. It was further purified by crystallization from an appropriate solvent (acetone, ethanol, benzene, etc.).

Diazomethane was prepared from *N,N'*-nitrosomethylurea using 1,4-dioxane in place of diethyl ether.[2] Schiff bases were prepared by heating equimolar amounts of the appropriate aldehydes and anilines in ethanol.[2]

Diazomethane is a toxic, mutagenic and explosive material. It should always be handled in a well-ventilated efficient hood and sharp or rough edges in glassware should be avoided. During the above preparation of diazomethane from nitrosomethylurea, the temperature of the reaction should be maintained at 8–10°C to avoid freezing of dioxane, which might otherwise result in an explosion. The mixture should be carefully introduced into a separatory funnel and the lower alkaline layer tapped out and diluted with water. The upper layer provides an aqueous dioxane solution of diazomethane, containing sufficient water to catalyze the reaction with Schiff bases, and must be used immediately.

The 1,2-diarylaziridines were prepared by photolysis of the 1,5-diaryltriazolines as shown in Fig. 1. The 1,2,3-triazolines generally absorb in the 310–390 nm region when irradiated with UV light, and undergo facile decomposition with expulsion of nitrogen to give either the pure aziridines, or mixtures containing minor amounts of imines depending on the substituent pattern of the triazolines.[6,15] In a typical photolysis reaction, the triazolines were irradiated in acetone solution using a 275W G.E. sunlamp which served as a convenient light source in the 300-nm range. The temperature of the acetone solution was maintained at 20–25°C during irradiation by the use of a 'cold finger' through which cold water was continuously circulated and which was kept immersed in the solution. The photodecomposition

TABLE 1
Aphicidal Activity of 1,2,3-Triazolines[a]

Compound number	*R*	*R'*	*Spray concentration (mg liter⁻¹)*	*Mortality after 72 h (%)*
1	*m*-NO_2	H	1000	0
2	H	*m*-NO_2	1000	0
3	H	*p*-NO_2	1000	0
4	*o*-Cl	H	64	88/100
5	*p*-Cl	H	1000	0
6	*o*-NO_2	*m*-NO_2	1000	0
7	*m*-NO_2	*m*-NO_2	1000	0
8	*p*-NO_2	*m*-NO_2	1000	0
9	*p*-NO_2	*p*-NO_2	1000	0
10	*m*-NO_2	*p*-Cl	500	0
11	*m*-NO_2	*p*-Br	1000	0
12	*p*-NO_2	*m*-Cl	1000	0
13	*p*-NO_2	*p*-Br	1000	0
14	*o*-Cl	*m*-NO_2	32	100/82
			16	0
15	*o*-Cl	*p*-Br	128	100
			32	24
16	*p*-Cl	*m*-NO_2	1000	0
17	*o*-NO_2	3,4-diCl	64	100
			16	47
18	*o*-Cl	3,4-diCl	16	96/100
			4	29
19	2,4-diCl	*m*-Cl	1000	0
20	2,4-diCl	*p*-Br	1000	0
21	2.4-diCl	3,4-diCl	1000	0

[a] See Fig. 1 for structure.

reaction proceeded at a relatively rapid rate and did not necessitate the use of an internal UV light source. Complete removal of the acetone in a rotary evaporator yielded aziridines **22–29** as clear viscous oils.

The aziridines were characterized by their typical NMR spectra.[16] Since the methylene protons of the 3-CH_2 group are diastereotopic,[6] the NMR spectra show two closely similar doublets for the 2-CH proton in the region δ 3–4 and a multiplet of eight peaks for the 3-CH_2 protons in the δ 2–3 region resembling an ABX system. This is distinctly different from the NMR spectra of the parent 1,2,3-triazoline compounds, where the 5-CH and 4-CH_2 protons are further downfield (δ 5–6) and appear as an ABC multiplet of 12 peaks.[6,17]

From the NMR spectra, the aziridine samples were estimated to contain 10–15% of the imine components, based on integration of the imine-CH_3 peak appearing as a sharp singlet in the δ 2–2·5 region along with the 3-CH_2 peaks of the aziridine ring. No attempt was made to remove these minor amounts of the imine, due to extensive decomposition even upon distillation under reduced pressure.

2.3 Ames mutagenicity test

Aziridine **24** was evaluated for potential mutagenic and carcinogenic properties using standard Ames assay procedures.[18] This assay used strains of *Salmonella typhimurium* (Loeffler) Castell. & Chalm. selected for sensitivity and specificity in being reverted from a histidine requirement back to prototrophy by a wide variety of mutagens. Potential changes in frameshift were evaluated using strain TA 98 and changes in base pairing were detected using strain TA 100. Three concentrations were tested which included 100, 500 and 1000 μg per plate using triplicate plates for each concentration. Tests were also conducted to examine if mutagens were produced during mammalian metabolism of **24** by employing arochlor-induced rat liver enzymes.

3 RESULTS

3.1 Screening results

Test results of 21 triazolines and eight aziridines for aphicidal activity are presented in Tables 1 and 2 respectively. The data indicated the presence of aphicidal activity in several 1,5-diaryl-1,2,3-triazolines, and this activity was found to be confined primarily to those compounds that have an *ortho*-chloro substituent on the C-phenyl ring, either alone (cf. **4** and **5**) or in combination with a *meta*-and/or a *para* substituent on the *N*-phenyl ring (cf. compounds **14**, **15** and **16**), with a combined *meta*, *para* substitution on the *N*-phenyl the most effective (cf.

TABLE 2
Aphicidal Activity of Aziridine Compounds[a]

Compound number	*R*	*R'*	*Spray concentration (mg liter^{-1})*	*Mortality after 48 h (%)*
22	*o*-Cl	*m*-NO_2	50	70
			10	50
23	*o*-Cl	*p*-Br	10	78
24	*o*-Cl	3,4-diCl	10	100
			5	66
25	*o*-Cl	*p*-Cl	10	81
26	*o*-Cl	*p*-F	10	17
27	*o*-Cl	*p*-CF_3	10	91
			5	33
28	*o*-Cl	*m*-Cl	50	100
			10	0
29	*o*-F	*p*-F	100	38

[a] See Fig. 1 for structure.

TABLE 3
Aphicidal Activity of 1-(3,4-Dichlorophenyl)-5-(2-chlorophenyl)-Δ^2-1,2,3-triazoline (**18**) With and Without UV Light Treatment

UV treatment	*Spray concentration (mg liter^{-1})*	*Mortality (%) after 18*	*24*	*48*	*72 h*
None[a]	16	8	25	14	14
	8	6	11	9	11
	4	3	3	6	7
Irradiated with	16	98	96	100	100
UV light of λ	8	68	75	87	84
354 nm	4	39	34	37	35
Control (vehicle)	—	9	12	13	12

[a] UV light was filtered through Mylar W_2.

15 to **18**). The nature of the *ortho* substituent group on the C-phenyl also affected activity to a large degree, with a halogen more effective than a nitro group (cf. **6** to **14** and **17** and **18**). However, introduction of an additional substituent in the *para* position of the C-phenyl ring eliminated activity. Thus, while triazoline **18** was highly active (96 to 100% control at 16 mg liter^{-1}), compounds having 2,4-substitution on the C-phenyl (**19**, **20** and **21**) were completely inactive.

The aphicidal activity of triazolines **14**, **15** and **18** (Table 1) correlated with the activity observed for the respective aziridines **22**, **23** and **24** (Table 2).

A comparison of the aphicidal activity of **18** and its dependence on UV light for activation is shown in Table 3. When the fluorescent light was filtered through Mylar W_2, the mortality rate of aphids for triazoline **18** decreased from 100% to 14% at 16 mg liter^{-1} and from 29% to 7% at 4 mg liter^{-1}. However, when the acetone solution of **18** that had been irradiated with UV light was tested in the absence of fluorescent light, there was an increase in aphid mortality to 100% at 16 mg liter^{-1}.

3.2 Ames assay

The standard Ames mutagenicity test for the aziridine **24** indicated that the latter was not mutagenic by itself or in the presence of mammalian liver enzymes (Table 4). Data showed no significant increases in the number of revertants per plate in the presence of the test compound, indicating that it did not cause mutations in DNA as the result of potential changes in frame shift and/or base pairing. At higher concentrations, some toxicity to bacteria was noticed in strain TA98.

Similarly, aziridines **23** and **25** had also been found non-mutagenic in separate tests.[19,20]

TABLE 4
Effect of Aziridine **24** on Two Strains of *Salmonella typhimurium*

Concentration (μg per plate)	*Strain TA 98*		*Strain TA 100*	
	Revertants per plate[a]		*Revertants per plate*[a]	
	Mammalian enzymes		*Mammalian enzymes*	
	Absent	*Present*	*Absent*	*Present*
0	65	80	149	191
100	44	50	148	214
500	42	55	150	202
1000	30	43	140	195

[a] All values are average of three plates.

4 DISCUSSION

The results presented in Table 3 clearly indicated that under identical experimental conditions, only in the presence of UV light did the triazoline **18** evince significant aphicidal activity; in the absence of UV light, no activity was observed. Since the triazolines are known to lose nitrogen under the influence of UV light,[6] to afford aziridines, it is logical to conclude that the aphicidal activity of the triazolines under fluorescent light (which contains UV light) was derived from the aziridines that were formed from the triazolines by photolysis. In fact, aziridines **22**, **23** and **24** (Table 2) corresponding to three of the most active triazolines **14**, **15** and **18** (Table 1), were found to possess even better aphicidal activity than the corresponding triazolines themselves.

Further supportive evidence is provided by the significant aphicidal activity found to be present in several related aziridine analogues **25–28** (Table 2). All contained an *o*-Cl substituent on the C-phenyl ring. A *p*-Cl substituent on the *N*-phenyl ring resulted in compounds with greater activity (**25**) than those with a *m*-Cl (**28**) or a *m*-NO_2 (**22**) group. On the other hand, a *p*-F substituent on the *N*-phenyl led to a decrease in activity, and aziridine **26** was the least potent among all the aziridine compounds tested. A further reduction in its activity was observed when the *o*-Cl on the C-phenyl was replaced by an *o*-F group (**29**). Aziridine **27** with a *p*-CF_3 on the *N*-phenyl was the most active among those analogues bearing only a single substituent group on the *N*-phenyl, and was comparable to the most active 3,4-diCl- substituted compound **24**.

The results presented in Table 2 also suggest certain Topliss–Hansch type structure–activity correlation patterns that relate to the lipid solubility (π) and electronic effects (σ) of the *N*-phenyl substituent groups.[21] With increasing $\pi + \sigma$ values (3,4-diCl = 1·77; *p*-CF_3 = 1·42; *p*-Br = 1·09; *p*-Cl = 0·94; *p*-F = 0·20), there was a corresponding increase in the aphicidal activity (**24** > **27** > **23** ~ **25** > **26** > **29**). It is remarkable that similar structure–activity relationship patterns have also been observed in the insecticidal activities of 15 different 1,2-diphenylaziridines on the 5th-instar Manduca sexta larvae, with the unsubstituted diphenylaziridine completely inactive.[19]

Other reports on the biological properties of aziridines include their antimicrobial activity[22,23] (aziridinyl benzoquinones and aziridine-1-carboxylic acid esters) and their use as fungicides and antibiotic and antitumor agents (e.g. mitomycins).[22] Although numerous aziridinyl phosphorous compounds (tepa, metepa, thiotepa apholate) possess chemosterilant activity towards a number of insect species,[22] they are highly toxic to mammals and are also carcinogenic[22] and mutagenic.[22,24–26] Aziridine-1-carboxylic acid amides also act as chemosterilants,[27,28] and 1-aryl-2-alkylaziridines are reported to be effective in controlling nematodes such as pinworms or ascarid worms.[29]

5 CONCLUSIONS

The significant aphicidal activity of the triazolines has been found to result from the aziridines formed *in situ* by the decomposition of the triazolines under the influence of the fluorescent lighting containing UV light. The observation of comparable or better aphicidal activity in the aziridines (**22**, **23** and **24**) corresponding to the three most active triazoline compounds (**14**, **15** and **18**), along with the observed activity for several additional structurally related aziridine analogues (**25–28**) supports this finding. The aziridine ring system bears structural resemblance to the cyclopropane ring, which is an integral part of the natural pyrethrins as well as of the synthetic pyrethroids. The –N– function of the aziridine ring is isosteric with the $-CH_2-$ group of the cyclopropane structure and isosteric replacements in biologically active compounds often permit the rational preparation of more selective compounds.[30] The aziridines reported here are not mutagenic in the Ames assay and thus provide a safer class of agricultural pesticides compared to chorinated hydrocarbons and organophosphates.

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REFERENCES

1. Kadaba, P. K. & Edwards, J. O., The action of diazomethane on Schiff's bases. *J. Org. Chem.*, **26** (1961) 2331–5.
2. Kadaba, P. K., Triazolines-II. Solvent effects on the 1,3-cycloaddition of diazomethane to Schiff bases and the synthesis of 1,5-diaryl-1,2,3-triazolines. *Tetrahedron*, **22** (1966) 2453–60.
3. Kadaba, P. K., Triazolines-IV. Solvation effects and the role of protic-dipolar aprotic solvents in 1,3-cycloaddition reactions. *Tetrahedron*, **25** (1969) 3053–66.
4. Kadaba, P. K. & Colturi, T. F., A kinetic investigation of the addition of diazomethane to styrenes. Role of dipolar aprotic solvents in predicting direction of dipole orientation. *J. Heterocycl. Chem.*, **6** (1969) 829–34.
5. Kadaba, P. K., Role of protic and dipolar aprotic solvents in heterocyclic syntheses via 1,3-dipolar cycloaddition reactions. *Synthesis*, 1973, 71–84.
6. Kadaba, P. K., Stanovnik, B. & Tisler, M., Δ^2-1,2,3-Triazolines. *Adv. Heterocycl. Chem.*, **37** (1984) 217–349.
7. Kadaba, P. K., Triazolines XIII. Δ^2-1,2,3-Triazolines, a new class of anticonvulsants. *J. Pharm. Sci.*, **73** (1984) 850–2.
8. Kadaba, P. K., Triazolines 14. 1,2,3-Triazolines and triazoles, a new class of anticonvulsants. Drug design and structure–activity relationships. *J. Med. Chem.*, **31** (1988) 196–203,
9. Mustafa, A., Action of diazomethane and diphenyldiazomethane on nitro-anils. *J. Chem. Soc.*, 1949, 234–6.

10. Buckley, G. D., Reaction of diazomethane with arylideneanilines. *J. Chem. Soc.*, 1954, 1850–1.
11. Kadaba, P. K., Triazolines VI. Evaluation of 1,5-diaryl-Δ^2-1,2,3-triazolines and arylideneanilines for herbicidal activity. *J. Pharm. Sci.*, **59** (1970) 1190–1.
12. Kadaba, P. K., Triazolines VII. Results of screening 1,5-diaryl-Δ^2-1,2,3-triazolines for pesticidal properties. *Pestic. Sci.*, **5** (1974) 255–8.
13. Kadaba, P. K. & Slevin, J. T., Triazolines XV. Anticonvulsant profile of ADD17014, a potentially unique 1,2,3-triazoline antiepileptic drug, in mice and rats. *Epilepsia*, **29** (1988) 330–7.
14. Kadaba, P. K., 1,2,3-Triazolines: A new generation of anticonvulsant agents effective in the kindling model of epilepsy, with a unique mechanism for impairing excitatory amino acid neurotransmission. *Drugs of the Future*, **15** (1990) 1013–24.
15. Scheiner, P. Photodecomposition of 1,2,3-Triazolines. A new entry into the aziridine series. *J. Org. Chem.*, **30** (1965) 7–10.
16. Liepin'sh, E. E., Pestunovich, V. A., Eremeev, A. V., Tikhomirov, D. A. & Gaidarova, N. P., Investigation of the conductivity of electronic effects by the aziridine ring. *Khim. Geterot. Soedin.* (*Chemistry of Heterocyclic Compds*) 1977, No. 7, 906 (Russ.); p. 731 (Engl.).
17. Kadaba, P. K., Triazolines VIII. Action of diazoalkanes on heterocyclic substituted Schiff bases. *J. Heterocycl. Chem.*, **12** (1975) 143–6.
18. Ames, B. N., McCann, J. & Yamasaki, E., Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Res.*, **31** (1975) 347–65.
19. Dahlman, D. L. & Kadaba, P. K., 1,2-Diphenylaziridines as pesticides. Studies on acute toxicity and delayed effects. *Pestic. Sci.* **22** (1988) 71–81.
20. Kadaba, P. K. & Dahlman, D. L., Pesticidal diphenylaziridines. US Patent 4 582 827, 1986.
21. Topliss, J. G. (ed.), Quantitative Structure–Activity Relationships of Drugs, Medicinal Chemistry Series, No. **19**. Academic Press, NY, 1983, pp. 1–21.
22. Dermer, O. C. & Ham, G. E., *Ethylenimine and other Aziridines*. Academic Press, New York, NY, 1969, pp. 340–443.
23. Pierce, A., *N*-Substituted aziridines for combating microorganisms. US Patent 3 487 157, Dec. 1969 (*C.A.*, **72** (1970) P77960).
24. Durham, W. F. & Williams, C. H., Mutagenic, teratogenic, and carcinogenic properties of pesticides. *Ann. Rev. Entomol.* **17** (1972) 123–48.
25. McCann, J., Choi, E., Yamasaki, E. & Ames, B. N., Detection of carcinogens as mutagens in the Salmonella/microsome test. Assay of 300 chemicals. *Proc. Natl. Acad. Sci.*, **72** (1975) 5135–9.
26. Quraishi, M. S., *Biochemical Insect Control*. Wiley-Interscience, New York, NY, 1977, p. 240.
27. Boston, M. D., Patterson, R. S. & Lofgren, C. S., Screening of chemosterilants against the southern house mosquito *Culex pipiens quinquefasciatus*. *Fla Entomol.*, **53** (1970) 215–18.
28. Crystal, M. M., Effects of delayed fertilization in screwworm flies on induction of dominant lethal mutations by *N,N'*-tetramethylenebis(1-aziridinecarboxamide). *Ann. Entomol. Soc. Amer.*, **63** (1970) 71–4
29. Kuhn, S. J., Trifluoromethyldinitrophenylaziridines. US Patent 3 297 682, Jan. 1967 (*C.A.* **66** (1967) P75902).
30. Daniels, T. C. & Jorgensen, E. C., In *Textbook of Organic Medicinal and Pharmaceutical Chemistry*, ed. C. O. Wilson, O. Gisvold & R. F. Doerge. J. B. Lippincott Co., Philadelphia, PA, 1977, p. 5.