were definitely paralyzed in less than 4 hours, while in strains 151, 127, and 150, 2 to 23 percent of the females survived for more than 24 hours. These results seem to explain the lack of residual effect in the stables.

Tests of resistance to parathion in several field strains (see Table 1) by topical application of the compound generally showed an LD_{50} of about 2 to 3 times normal in each strain. In strain 79 from a farm where parathion has been used as a residual spray for at least 2 years, the parathion resistance was about 5 times normal.

The practical significance of this resistance is not yet quite clear. On most farms the parathion-strips still seem to work well when used according to our directions. However, preliminary tests indicated that some resistant flies (strains Nos. 74 and 79) required a contact period on the parathion strips about 4 times longer than did the laboratory strains.

In conclusion, our results with Bayer 21/199 and Diazinon have shown that development of resistance may impede the control of houseflies even with organic phosphorus compounds. So far, the future use of this group of insecticides for fly control has been regarded with optimism in view of the apparently modest resistance (LD_{50} 10 to 20 times normal) obtained by long-term selection in the laboratory (*3*). However, as is shown in this report, an increase in tolerance of only 10 times may be of significance for the control of flies with residual sprays (*4*).

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References and Notes

1. J. Keiding, *Annual Rept. Statens Skadedyrlaboratorium* 1951–52 (1954), p. 24; and 1952–53 (1955), p. 28.
2. H. Wichmand, *Nature* 172, 758 (1953).
3. E. F. Knipling, *1st Intern. Symposium on Control of Insect Vectors of Disease Rend. 1st Super. Sanita, Suppl.* (1954), p. 318.
4. Further investigations are in progress, and a more detailed report is in preparation.

9 February 1956

Inhibition of Enzymatic Browning of Chlorogenic Acid Solutions with Cysteine and Glutathione

Weurman and Swain (*1*) have demonstrated that chlorogenic acid is one of the substrates involved in the enzymatic browning of both apple and pear homogenates and that three new fluorescent compounds were formed during the reaction. Work in this laboratory (*2*) with apples confirms the disappearance of chlorogenic acid in aerated homogenates as well as during the development of storage scald, but no new fluorescent spots were observed on paper chromatograms of the browned solutions. However, further studies have resulted in evidence for the formation of fluorescent *o*-quinone-sulfhydryl addition complexes.

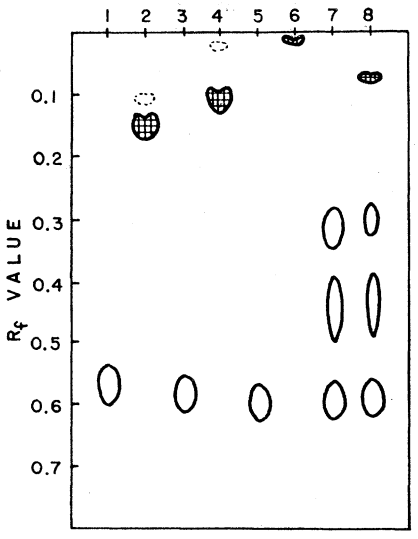

Fig. 1. Chromatographic patterns of fluorescent compounds found after 1 hour of aeration in various reaction mixtures: (i) apple enzyme + chlorogenic acid + cysteine, boiled, *1*, and unboiled, *2*; (ii) apple enzyme + chlorogenic acid + glutathione, boiled, *3*, and unboiled, *4*; (iii) apple enzyme + chlorogenic acid + coenzyme A, boiled, *5*, and unboiled, *6*; (iv) cabbage extract, boiled, *7*, and unboiled, *8*.

In this study, dilute aqueous extracts of Grimes Golden apple tissue, pulp and peel, were used as a source of polyphenoloxidase. Enzyme solutions were prepared by macerating 25 g of tissue with 200 ml of distilled water for 2 minutes in a Waring Blendor, allowing the solids to settle, and decanting the liquid portion. Inactive enzyme solutions were prepared by macerating the tissue in boiling water. When 1 drop of a concentrated chlorogenic acid solution was added to 1-ml aliquots of both the active and inactive enzyme solutions, rapid browning was observed in the aliquots containing the active enzyme and no browning in the aliquots containing the inactive enzyme. After 1 hour, approximately 100-lambda aliquots were spotted on Whatman No. 1 filter paper and separated by descending chromatography with the organic phase of butanol - acetic acid - water (4 : 1 : 5). Examination of the dried chromatograms under ultraviolet light revealed an intense blue-white fluorescent spot (R_f = 0.57) corresponding to chlorogenic acid in the nonbrowned, inactive enzyme solution. Neither chlorogenic acid nor any new fluorescent products of the browning reaction were detected on chromatograms of the browned, active enzyme solutions. The addition of ascorbic acid to mixtures of chlorogenic acid and active apple enzyme inhibited browning as well as the disappearance of the chlorogenic acid spot on the chromatograms. Ascorbic acid is known to reduce quinones back to phenols (*3*) and also to have an inhibitory effect on the enzyme (*4*).

The inhibition of browning reactions of chlorogenic acid with cysteine or glutathione appears to proceed by a different mechanism. The addition of cysteine or glutathione to a mixture of chlorogenic acid and active apple enzymes resulted in inhibition of browning; as may be seen in Fig. 1, there is a complete disappearance of chlorogenic acid and the appearance of a new, yellow fluorescent spot (R_f = 0.16 for cysteine-chlorogenic acid; R_f = 0.11 for glutathione-chlorogenic acid). The coenzyme-A solution tested did not prevent browning, but a new, faint yellow fluorescent spot (R_f = 0.02) appeared on the chromatogram. Since the sulhydryl content of this preparation was not known, the data only support the suggestion that coenzyme A participates in these reactions. The chromatographic spots of the cysteine- and glutathione-chlorogenic acid complexes gave an immediate reddish-brown color on spraying with ammoniacal silver nitrate, a blue color with ninhydrin, and a white spot against a pink background with the KI-H_2PtCl_6 reagent for sulfur compounds (*5*). Cysteine (R_f = 0.04) and glutathione (R_f = 0.20) were also detected on the chromatograms by the latter two spray reagents. These data indicate that these sulfhydryl compounds inhibit *in vitro* browning by removal of the quinone produced by polyphenoloxidase from further participation in the browning reaction. The nonsulfhydryl amino acids—glycine, alanine, and glutamic acid—did not inhibit browning. Glutamic acid did not appear to increase

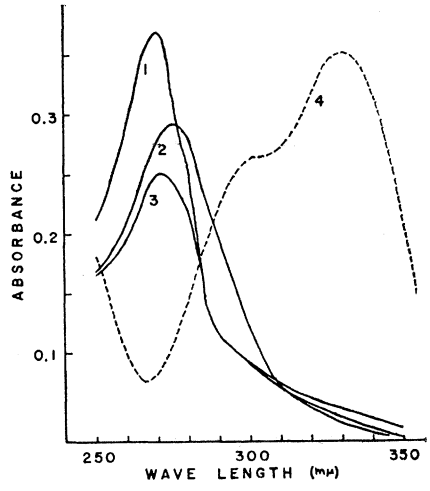

Fig. 2. Ultraviolet absorption spectra of the addition products of various sulfhydryl compounds with the quinone of chlorogenic acid: *1*, aerated cabbage compound; *2*, cysteine addition product; *3*, glutathione addition product; *4*, chlorogenic acid alone.

browning as reported by Shiroya and Hattori (*6*). Thioglycollic acid inhibited browning with no loss of chlorogenic acid. Thiourea inhibited browning with a loss of chlorogenic acid and the appearance of a new spot; this suggests a function other than chelation of copper (*7, 8*).

Extracts of cabbage were also tested. After standing for 1 hour, the extract did not brown, but as is shown in Fig. 1, a new, yellow fluorescent spot ($R_f = 0.08$) appeared on the chromatogram from the nonboiled cabbage extract. This spot gave an immediate reddish-brown color after it had been sprayed with ammoniacal silver nitrate, and it gave a faint test for sulfur with the KI-H_2PtCl_6 reagent. A definite ninhydrin test was obscured by the abundance of free amino acids in the extract. Chlorogenic acid and two other bluish-white fluorescent areas were evident in both the boiled and nonboiled cabbage extracts.

Figure 2 shows the ultraviolet absorption spectra of alcohol solutions of the cysteine-, glutathione- and cabbage-chlorogenic acid compounds obtained after extracting their corresponding yellow fluorescent areas from paper chromatograms.

In view of the widespread occurrence of *o*-phenolic substrates and polyphenoloxidase in plant tissues, the possible *in vivo* operation of these reactions requires further study.

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References and Notes

1. C. Weurman and T. Swain, *Nature* 172, 678 (1953).
2. This report is journal paper No. 855 of the Purdue University Agricultural Experiment Station. Appreciation is expressed to H. M. Barnes, General Foods Corp., for a sample of authentic chlorogenic acid.
3. L. W. Mapson, in *The Vitamins*, W. H. Sebrell and R. S. Harris, Eds. (Academic, New York, 1954), vol. 1, p. 224.
4. P. Baruah and T. Swain, *Biochem. J. London* 55, 392 (1953).
5. H. M. Winegard, G. Toennis, R. J. Black, *Science* 108, 506 (1948).
6. M. Shiroya and S. Hattori, *Physiol. Plantarum* 8, 358 (1955).
7. L. E. Tenenbaum and H. Jensen, *J. Biol. Chem.* 147, 27 (1943).
8. H. Heymann, Z. Rogach, R. L. Mayer, *J. Am. Chem. Soc.* 76, 6330 (1954).

13 February 1956

Systemic Control of Cherry Leaf-Spot Fungus by Foliar Sprays of Actidione Derivatives

In an evaluation of the antibiotic actidione (cycloheximide) on potted Montmorency cherry trees in the greenhouse, it was found that this material is highly specific in fungicidal activity against the cherry leaf-spot fungus, *Coccomyces hiemalis* Higgins, not only as a protectant, but also as an eradicant of the fungus after it is established in the leaf [J. M. Hamilton and M. Szkolnik, *Phytopathology* **43**, 109 (1953)]. The testing of certain derivatives of actidione for their eradicative activity during the 1954 season led to the discovery that the oxime derivative is effective systemically in the control of the cherry leaf-spot fungus.

Table 1. Control of cherry leaf spot systemically with the oxime derivative of actidione.

Treatment	Interval between spraying and inoculation	Leaf spot on new leaves: Lesions per 2 in.²*
	Series A, 26/July/54	
Actidione 1 ppm	2 weeks	83 ±
Oxime 10 ppm	2 weeks	20
Oxime 60 ppm	2 weeks	Trace
Oxime 10 ppm + lime 2–100	2 weeks	42
Oxime 60 ppm + lime 2–100	2 weeks	19
	Series B, 11/Apr./55	
Unsprayed	1 week	49
Actidione 1 ppm	1 week	50
Oxime 30 ppm	1 week	17
Oxime 60 ppm	1 week	4
	Series C, 12/May/55	
Unsprayed	3 weeks	39
Oxime 30 ppm	3 weeks	20
Oxime 60 ppm	3 weeks	4
	Series D, 27/Oct./55†	
Unsprayed	5 days	69
Oxime 10 ppm	5 days	39
Oxime 60 ppm	5 days	13
Semicarbazone 10 ppm	5 days	2
Isomer 10 ppm	5 days	1
Acetate 10 ppm	5 days	5
Anhydro 10 ppm	5 days	17
Dehydro 10 ppm	5 days	32

* Average of four heaviest infected new leaves per shoot.
† Averaged for the two to three new leaves per shoot. The derivatives of series D were prepared by the Upjohn Co., Kalamazoo, Mich.

Greenhouse-grown cherry trees were sprayed with the test materials, kept in the greenhouse for the development of new growth, and inoculated with *C. hiemalis* 2 to 3 weeks later. Following inoculation, the trees were placed in a saturated humidity chamber for 48 hours at 18.5°C to provide conditions suitable for infection by the fungus. The trees were then returned to the greenhouse for completion of the incubation period.

It was found that the oxime at 60 ppm was taken up by sprayed foliage and translocated to new growth in sufficient amounts to provide protection against infection by the leaf-spot fungus (Table 1). Actidione does not exhibit this systemic activity. The systemic protection by the oxime extended through the seven to nine new leaves that grew during the 2- to 3-week interval between initial spraying and subsequent inoculation. There was a significant reduction of shoot growth with the oxime derivative at concentrations greater than 60 ppm, but injury is not considered a serious factor at the 60-ppm level. The oxime derivative lost part of its stability in the presence of hydrated spray lime.

The oxime derivative at 40 ppm was effective in inhibiting further development of the cedar-apple rust, *Gymnosporangium juniperi-virginianae* Schw., in the foliage of potted Rome Beauty trees when it was applied 24 hours after inoculation or when the lesions were just becoming visible.

Inasmuch as the semicarbazone derivative of actidione also gave indication of being systemically active in early tests, other derivatives of actidione were evaluated in 1955. The isomer, acetate, and semicarbazone derivatives were found to be definitely systemic at 10 ppm or lower concentrations, which are within the economical level for agricultural use. The anhydro derivative also exhibited systemic activity but not as much as that of the three derivatives just mentioned. Injury with these derivatives at 10 ppm or lesser concentrations was of no significance in the greenhouse tests but ranged from moderate to severe at 60 ppm. Among the factors to be explored more fully are the interrelationship of concentration, the number of leaves sprayed for chemical uptake, and the extent of new growth to be protected.

The isomer, acetate, and semicarbazone derivatives were protective against the leaf-spot fungus at 0.5 ppm and gave good eradicative control at 1 ppm when they were applied 4 days after inoculation.

It has not yet been determined whether these derivatives remain active as such within the plant tissue or whether they are altered through some biochemical