was a tendency toward larger yields (not significant) from plots treated 1 week before harvest.

It was concluded that malathion applied 2 weeks before harvest at 1600 ppm and at a rate equivalent to 100 gal/acre, would give good color enhancement and still be within the label restrictions for the use of this material on cranberries.

LITERATURE CITED

1. CLOWER, D., and J. MATTHYSSE. 1954. Phytotoxicity of insecticides in mist concentrate type formulations. *J. Econ. Ent.* 47:735–738.
2. DEHERTOGH, A. A., D. C. MCCUNE, J. BROWN, and D. ANTOINE. 1965. The effect of antagonists of RNA and protein biosynthesis on IAA and 2,4-D induced growth of green pea stem sections. *Contrib. Boyce Thompson Inst.* 23:23–31.
3. DEVLIN, R. M. 1967. Chemical inducement of anthocyanin formation in *Vaccinium macrocarpon* (var. Early Black). *Ann. Meeting Amer. Soc. Hort. Sci.* 64:64–65.
4. ————, B. M. ZUCKERMAN, and I. E. DEMORANVILLE. 1968. Effect of delayed harvest and storage on the pigment development in cranberries. *Proc. Amer. Soc. Hort. Sci.* 92:793–796.
5. EBERHARDT, F. 1955. Der Atmungsverlauf alternader und reifer Früchte. *Planta* 45:57–67.
6. ECK, P. 1968. Chemical color enhancement of cranberry fruit. *HortScience* 3(2):70–72.
7. FRANCIS, F. J., and W. M. ATWOOD. 1961. The effect of fertilizer treatments on the pigment content of cranberries. *Proc. Amer. Soc. Hort. Sci.* 77:351–358.
8. FUDGE, B. R. 1930. Increasing the color of cranberries after removal from the vines. *N. J. Agr. Exp. Sta. Bul.* 504:3–13.
9. FULEKI, T., and F. J. FRANCIS. 1968. Quantitative methods for anthocyanins. I. Extraction and determination of total anthocyanin in cranberries. *J. Food Sci.* 33:72–77.
10. GALSTON, A. W., and W. K. PURVIS. 1960. The mechanism of action of auxin. *Ann. Rev. Plant Physiol.* 11:239–276.
11. HAVILAND, E., and H. HIGHLAND. 1955. The phytotoxicity of malathion to poinsettias. *J. Econ. Ent.* 48:326.
12. MASUDA, Y., E. TANIMOTO, and S. WADA. 1967. Auxin-stimulated RNA synthesis in oat coleoptile cells. *Physiol. Plant.* 20:713–719.
13. NEISH, A. C. 1964. Major pathways of biosynthesis of phenols. *In* Biochemistry of Phenolic Compounds, J. B. Harborne (ed.). Academic Press, N. Y.
14. SACHER, J. A. 1967. Senescence: action of auxin and kinetin in control of RNA and protein synthesis in subcellular fractions of bean endocarp. *Plant Physiol.* 42:1334–1342.
15. SERVADIO, G. J., and F. J. FRANCIS. 1963. Relation between color of cranberries and color and stability of sauce. *Food Tech.* 17:124–128.
16. STAFFORD, H. A. 1966. Regulatory mechanisms in anthocyanin biosynthesis in first internodes of *Sorghum vulgare:* effect of presumed inhibitors of protein synthesis. *Plant Physiol.* 41:953–961.
17. THIMANN, K. V., and B. S. RADNER. 1955. The biogenesis of anthocyanins. IV. The inhibiting effect of methionine and other sulphur containing compounds on anthocyanin formation. *Arch. Biochem. Biophys.* 2:484–497.
18. ZANONI, G. 1938. La pigmentazione antocianica quale indice di tipi costituzionali. *Boll. Mus. Lab. Zool. Anat. Comp. R. Univ. Genova* 18:51–63.
19. ————. 1938. Metabolismo di base e costituzione nelle forme antocianiche. *Boll. Mus. Lab. Zool. Anst. Comp. R. Univ. Genova* 18:65–87.

Some Biochemical Effects in Apple Leaf Tissue Associated with the Use of Simazine and Amitrole[1]

Maria Solecka, Hanna Profic and D. F. Millikan,
University of Missouri, Columbia

Abstract. Simazine at both 4 and 8 lb./A and amitrole at 2 lb./A, alone and in combination, altered the protein and RNA content of apple leaf tissue. Initially, simazine increased the protein content, but this effect progressively diminished until late June when it became nonexistent. Similarly, the use of amitrole caused an early season increase in protein content, later changing to a marked decrease. Greatest effects occurred in the total and globulin fractions while the soluble fraction was affected only slightly and this was limited to the first 6 weeks of growth.

Total RNA also was affected. This effect occurred in mid-May when simazine was stimulatory and amitrole acted as a depressant. These same effects persisted until late June when simazine at 4 lb./A and amitrole strongly decreased the RNA content. Enhanced action due to the simultaneous use of both chemicals did not occur, but there was evidence that the depressing effect due to amitrole was moderated when used with simazine, particularly in the period when shoot expansion ceased.

INTRODUCTION

INCREASING use of herbicide chemicals has created some apprehension concerning the possible residual effects, particularly with such chemicals as the triazines which possess marked persisting properties. Some experimental evidence indicated that their use promoted beneficial effects in the case of young apple and peach trees (4) wherein increases in leaf proteins have been observed. It also has been demonstrated that simazine, diuron and amitrole can be used at 3 to 4 times the usual rates on young apple trees with no apparent injury (6).

In 1965, a local nursery noted several unusual effects, apparently associated with the use of simazine and amitrole. One planting of spur type 'Red Delicious' developed a nice branching habit, contrasting with the usual single stem whips. Another group of trees exhibited symptoms resembling those of the rubbery wood virus (3). Such observations indicated a need for examining the use of these chemicals under more clearly defined conditions. We were unable to reproduce any of these observed abnormalties, but certain physiological changes were evaluated by measuring the effects upon the RNA and protein fractions.

MATERIALS AND METHODS

Spur type 'Red Delicious' trees were obtained from a nursery and set out in early March in 3 groups of 6 trees, each group of trees containing 6 treatments. Simazine at 4 and 8 lb./A and amitrole at 2 lb./A, alone and in combination, were applied on April 1, 1966. These treatments followed a modificated Latin square type of design, but the trees developed such poor growth during the unfavorable 1966 growing season, that no leaves were collected. On April 1, 1967, the chemical treatments were repeated and subsequent collections of leaf tissue were made at appropriate intervals. The first collection was made on April 11 when 4 to 5 fully expanded leaves appeared on the terminals. Similarly aged tip leaves were harvested periodically until growth ceased in late June. The harvested leaves were lyophilized and stored as dry leaf powder until used. Total protein was estimated on the leaf powder by a micro-Kjeldahl method. Soluble proteins were extracted with borate buffer, pH 8.3 (9) and measured ac-

[1]Received for publication April 27, 1968. Journal Series No. 5312. Approved by the Director of the Missouri Agricultural Experiment Station. This work was supported in part by a grant, ES 00082-16, from the Bureau of State Services.

Table 1. Effect of simazine and amitrole upon the total leaf protein of a spur type 'Red Delicious' apple.[x]

Treatment[y]		Total protein from leaf tissue collected on			
Herbicide	lb./A	April 11, 1967	May 12, 1967	June 1, 1967	June 28, 1967
none		10.87^{a}	8.27	6.99^{a}	7.28^{abd}
simazine	8	13.26^{b}	9.65^{abc}	8.49^{bc}	7.11^{abcd}
simazine	4	11.01^{a}	9.64^{abc}	8.29^{bcd}	7.57^{ab}
amitrole	2	12.11^{c}	9.04^{ab}	6.94^{a}	6.13^{cd}
simazine + amitrole	8 + 2	13.18^{b}	10.26^{ac}	7.95^{cde}	6.27^{acd}
simazine + amitrole	4 + 2	11.21^{a}	9.82^{ac}	7.60^{de}	6.99^{abcd}

[x]Data expressed in mg/g dry wt (% dry wt = mg/g dry wt $\times 10^{-1}$).
[y]Means within an experimental column followed by the same letter(s) are not significantly different at the 5% probability level.

Table 2. Effect of simazine and amitrole upon the globulin protein of a spur type 'Red Delicious' apple.[x]

Treatment[y]		Gloublin protein from leaf tissue collected			
Herbicide	lb./A	April 11, 1967	May 12, 1967	June 1, 1967	June 28, 1967
none		4.16^{a}	2.68^{a}	4.19^{a}	4.27^{b}
simazine	8	4.34^{b}	2.80^{a}	3.99^{b}	4.93^{b}
simazine	4	4.74^{b}	2.88^{ab}	3.72^{c}	4.61^{b}
amitrole	2	4.11^{b}	2.86^{a}	3.27^{c}	3.75^{b}
simazine + amitrole	8 + 2	4.79^{b}	3.13^{b}	4.06^{ab}	3.99^{a}
simazine + amitrole	4 + 2	4.18^{a}	2.84^{a}	4.12^{ab}	4.03^{a}

[x]Data expressed in mg/g dry wt (% dry wt = mg/g dry wt $\times 10^{-1}$).
[y]Means within an experimental column followed by the same letter(s) are not significantly different at the 5% probability level.

cording to Lowry et al. (2) Globulin proteins were considered as those precipitating with 50% $(NH_4)_2SO_4$ and quantitatively determined by the Lowry technique. Total RNA was extracted and determined spectrophotometrically upon alkaline hydrolysates after purification by passage over Dowex AG 1-X8 column (5). Data then were subjected to analysis of variance and Tukey's multiple means comparison's tests (7) for statistical significance.

Results

There was a significant treatment effect upon the total protein in samples of all leaf tissue collected in the study (Table 1). In the first collection, this effect was observed as an increased protein for both treatments containing 8 lb./A of simazine. Treatments with the lower amounts of simazine did not vary from the non-treated controls. In May, all chemical treatments increased the total protein and, aside from the amitrole-only treatment, this was also true in early June. By late June the only chemical affect was a decreased total protein found in the treatment with amitrole alone.

Leaf globulin protein also reflected a significant treatment affect in all of the periods under observation (Table 2). In April all treatments except simazine at 4 lb./A plus amitrole increased the globulin protein. Four weeks later only the simazine at 8 lb./A plus amitrole increased the globulin protein. By early June all 3 single chemical treatments were responsible for decreased globulin protein while the combination treatments caused no quantitative changes. In late June all treatments had globulin protein content quantitatively different from that found in the non-treated controls. Both treatments involving only simazine increased the globulin protein while the others decreased it. Greatest decrease was associated with amitrole by itself, the combination treatments reflecting a moderating influence on the effect of simazine.

Soluble proteins were affected less than the other protein fractions, significant treatment affects being restricted to the first two collections (Table 3). In April, simazine at the 4 lb./A and simazine at the higher rate plus amitrole both increased the protein. In May these treatments and other combination treatment increased the soluble proteins.

Total RNA was affected in all periods, but this effect followed a pattern differing from that observed in the protein fractions (Table 4). Initially only the simazine at 4 lb./A plus amitrole affected the total RNA content, causing an increase. By May, however, simazine at 8 lb./A and both combination treatments increased total RNA, whereas, amitrole exerted a depressing affect. In early June, all treatments influenced the total RNA and, except for amitrole and amitrole plus 4 lb./A simazine, all treatments did not differ from each other. Simazine at 4 lb./A and 8 lb./A as well as simazine at the lower rate plus amitrole, increased the total RNA while amitrole by itself exerted a depressing affect. By late June, all treatments except simazine at the higher rate, decreased the RNA content.

Table 3. Effect of simazine and amitrole upon the soluble proteins of a spur type 'Red Delicious' apple.[x]

Treatment[y]		Soluble protein from leaf tissue collected on			
Herbicide	lb./A	April 11, 1967	May 12, 1967	June 1, 1967	June 28, 1967
none		6.13^{a}	4.11^{ac}	5.53^{a}	5.02^{a}
simazine	8	6.13^{a}	4.18^{ac}	5.37^{a}	5.13^{a}
simazine	4	6.86^{b}	4.39^{bc}	5.30^{a}	5.82^{a}
amitrole	2	6.19^{a}	4.03^{ac}	4.38^{a}	4.27^{a}
simazine + amitrole	8 + 2	7.18^{b}	4.80^{d}	5.33^{a}	4.55^{a}
simazine + amitrole	4 + 2	6.05^{a}	4.20^{abc}	4.74^{a}	5.05^{a}

[x]Data expressed in mg/g dry wt (% dry wt = mg/g dry wt $\times 10^{-1}$).
[y]Means within an experimental column followed by the same letter(s) are not significantly different at the 5% probability level.

Table 4. Effects of simazine and amitrole upon the total RNA in leaf tissue of a spur type 'Red Delicious' apple.[x]

Treatment		RNA in leaf tissue collected			
Herbicide	lb./A	April 11, 1967	May 12, 1967	June 1, 1967	June 28, 1968
none		15.11^{a}	15.03^{ab}	14.23^{b}	15.39^{a}
simazine	8	15.36^{a}	16.49^{b}	17.56^{b}	15.74^{a}
simazine	4	15.27^{a}	15.74^{c}	15.82^{b}	13.59^{b}
amitrole	2	14.69^{a}	14.38^{a}	12.97^{a}	11.30^{c}
simazine + amitrole	8 + 2	14.79^{a}	18.93^{d}	15.18^{b}	11.82^{c}
simazine + amitrole	4 + 2	15.56^{b}	16.46^{b}	13.39^{a}	13.01^{b}

[x]Data expressed in mg/g dry wt (% dry wt = mg/g dry wt $\times 10^{-1}$).
[y]Means within an experimental column followed by the same letter(s) are not significantly different at the 5% probability level.

Discussion

These data show that both simazine and amitrole can alter the protein content of apple leaf tissue collected periodically from the time when the first fully differentiated leaves are formed until the period of rapid growth ceases. This effect was restricted primarily to the early part of the growing season and diminished or disappeared when the period of rapid shoot elongation is completed.

The observed variability in total protein, due to chemical stress seemed to be due primarily to effects upon the globulin fraction. This was indicated by the similar patterns of variability found in the residual soluble fraction.

Both simazine and amitrole affected the total RNA content, but this effect was not pronounced until the second period and, except for simazine at the higher rate, persisted throughout the study. Indications that amitrole does affect RNA were suggested in the studies of Carter and Naylor (1) who reported reductions in the free pool of the amino acids, glycine and serine, in beans treated with amitrole. Both of these amino acids are recognized as precursors of RNA. Sund et al. (8) gave additional evidence in their investigations, wherein they were able to alleviate the growth inhibition in amitrole treated tomato with adenine, guanine and hypoxanthine.

Our data also show that deleterious synergistic effects resulting from simultaneous use of simazine and amitrole did not occur. Some interaction was observed, but this appeared to be beneficial since simazine seemed to counteract some of the depressing influence of amitrole.

Literature Cited

1. Carter, M. C., and A. W. Naylor. 1961. The effect of 3-amino-1,2,4-triazole upon the metabolism of carbon labeled sodium bicarbonate, glucose, succinate, glycine and serine by bean plants. *Physiol. Plant.* 14:62–71.
2. Lowry, O. H., N. H. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:225–272.
3. Luckwill, L. C., and S. H. Crowdy. 1950. Virus diseases of fruit trees. II. Rubbery wood, chat fruit and mosaic in apples. *Ann. Rep. Long Ashton Res. Sta.* 1949:68–79.
4. Ries, S. K., R. P. Larsen, and A. L. Kenworthy. 1963. The apparent influence of simazine on nitrogen nutrition of peach and apple trees. *Weeds* 11:270–273.
5. Millikan, D. F., and E. E. Pickett. 1964. A rapid method for the extraction and identification of RNA in plant leaf tissue. *Phytochem.* 3:667–670.
6. Saidak, W. J., and W. M. Rutherford. 1963. The tolerance of young apple trees to amitrole, diuron and simazine. *Can. J. Plant Sci.* 43:113–118.
7. Snedecor, G. W. 1956. Statistical Methods. Ed. 5. Iowa State College Press, Ames, Iowa. 534 p.
8. Sund, K. A., E. C. Putala, and H. N. Little. 1960. Reduction of 3-amino-1, 2,4-triazole phytotoxicity in tomato plants. *J. Agric. Food Chem.* 8:210–212.
9. Zucker, M., and H. T. Stinson, Jr. 1962. Chloroplasts as the major protein bearing structures in *Oenothera* leaves. *Arch. Biochem. Biophys.* 97:637–644.

Pre-emergence Weed Control in Young Deciduous Fruit Trees[1]

A. H. Lange,[2] J. C. Crane,[3] W. B. Fischer,[4] K. O. Roberts[4] and C. L. Elmore,[2,5]
University of California

Abstract. A number of pre-emergence soil residual herbicides were tested at 2 locations on varieties of young peach, plum, cherry, pear and walnut rootstocks. The greatest variation in response resulted from differences in location. Important differences in varietal response were also obtained with the various herbicides in light soils. Simazine appeared sufficiently safe to trees in heavier soil but gave variable weed control. Diuron gave about the same degree of weed control but more safety than simazine on young trees. Of the uracil herbicides tested, DP-733 was the least toxic to the fruit tree species tested, while bromacil and isocil were generally the most toxic, except to peach trees. Of the commercial uracil herbicides, only DP-732 (terbacil) was of sufficient interest for further study.

Introduction

WEED competition in the culture of deciduous orchards is most damaging during the first few years in the establishment of young trees. Most preemergence herbicides, labeled for use in orchards, restrict the use of soil herbicides for trees older than 3 years. The object of this study was to evaluate some of the more promising herbicides for preemergence weed control in orchards with young deciduous fruit trees.

A number of studies have been conducted on young deciduous fruit trees (3, 7). Benson and Degman (1) reported good weed control with diuron on young apple and pear trees, but insufficient safety with simazine. Larsen and Ries (6) reported adequate safety with simazine on apples but less safety on young pear trees. Fischer (2) reported good safety with isocil and bromacil on young peach trees. Trifonov (9) stated that simazine should not be used on the seed bed, or on 1- to 2-year-old apple, pear, plum, quince, peach, cherry and apricot trees.

The results reported here are a summary of 3 tests of a number of preemergent type herbicides on 9 different species of young deciduous fruit trees. These trials were conducted in 3 widely different climatic areas and soil conditions. The effects of the herbicide treatments were measured by the foliar conditions and growth measurements of the trees.

Materials and Methods

Young commercial nursery trees were lined out one foot apart in small 5 × 10-foot plots and treated shortly thereafter with a number of herbicides in aqueous solutions at 100 gal/A. During the application, no attempt was made to keep the herbicide spray off the dormant trees. At various periods during the spring and summer, foliar conditions were rated for phytotoxicity (0 = no effect, 3 = definite recognizable symptoms related to the herbicide, 5 = a striking symptom with marginal burn, 10 = all foliage dead). Tree diameters and circumferences were taken at all 3 locations. Fresh weights showed more variation than diameters and were therefore not included. Trees at the Kearney trial grew poorly and showed no significant differences in diameter; therefore only foliar phytotoxicity ratings are listed.

The herbicides used were simazine [2-chloro-4,6 bis (ethylamino)-*s*-triazine], atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), prometryne (2,4-bis(isopropylamino)-6-methylmercapto-*s*-triazine), diuron [3-(3, 4-dichlorophenyl)-1,-1-dimethylurea], isocil [5-bromo-3-isopropyl-6-methyluracil], bromacil [5-bromo-3-*sec*-butyl-6-methyluracil], terbacil (3-*Tert*-butyl-5-chloro-6-methyluracil), DP629 (5-

[1]Received for publication March 20, 1968.

[2]Weed Control Specialist, Agricultural Extension Service, Riverside and Davis, respectively.

[3]Pomologist, Pomology Department, Davis.

[4]Farm Advisors, Agricultural Extension Service, Fresno and Sonoma Counties, respectively.

[5]The authors wish to thank the Geigy Chemical Company for financial support of this work, and also George McCall of the E. I. duPont de Nemours and Company for his help in the Sonoma trial. They acknowledge the technical assistance of Jim Yeager, Lee Smith and James Hicks of the University of California, Davis; Frank Domeline of the Sonoma County staff; the staffs of the Pomology Department, UCD and the Kearney Field Station. The statistics were run by Dr. T. M. Little, Biometrician, University of California, Riverside.