

explained is the lag between apparent early deamination, incident to the rapid production of yeast protein, and the later appearance of the higher alcohols. This suggests that some intermediate steps between amino acid deamination and formation of the corresponding alcohols proceed more slowly than the earliest steps in assimilation. Neubauer and Fromherz (2) demonstrated that the most likely early intermediates were ketonic acids, and not hydroxy acids as previously supposed by Ehrlich (1, 10). These ketonic acids are later decarboxylated, according to Neubauer and Fromherz, to form aldehydes which are subsequently reduced to alcohols. These facts, together with the lag between amino acid assimilation and the appearance of fusel oil, the concurrence between ethanol and fusel oil formation, and the simultaneous cessation of both processes found in our experiments, suggest a simple explanation of the relationship between conversion of sugar to ethanol and the formation of fusel oil. It is likely that the final steps of both these metabolic processes make use of one or more identical enzyme systems. The slow appearance of the higher alcohols could be explained by competition between large amounts of sugar fermentation intermediates and small amounts of fusel oil intermediates, for the carboxylase and the hydrogen-transferring alcohol dehydrogenase-Coenzyme I systems of the zymase complex. When the supply of sugar fermentation intermediates which yield hydrogen to Coenzyme I failed because of the disappearance of sugar, the hydrogen transfer system could no longer reduce the aldehyde precursors of fusel oil components. Thus the formation of both ethanol and higher alcohols would simultaneously be terminated.

The appearance of the excess yield of fusel oil, which in our experiments greatly exceeded the theoretical yield based on the actual amount of leucine, isoleucine, and valine that disappeared, raised a question as to the source of precursors. A good possibility lies in the statements of Ehrlich (1) and Harden (11), that yeast can form fusel oil at the expense of its own protein when the extracellular supply of suitable organic nitrogenous nutrients is negligible. Freshly cultured yeast cells, when prevented from rapidly multiplying under conditions which still permit vigorous enzymatic activity, may be considered to be undergoing autolysis. Among the products of autolytic breakdown of yeast protein under such conditions there should be amounts of the specific amino acid precursors of fusel oil. There is some evidence for this in our experiments—namely, a measurable increase in the residual amounts of isoleucine, leucine, and valine in the medium during the stage of alcoholic fermentation subsequent to the cessation of rapid yeast multiplication (Fig. 1).

Other possibilities of accounting for the formation of fusel oil in excess of the theoretical yield have been considered, but such possibilities involve the abandonment of the Ehrlich mechanism as a major factor in fusel oil formation. The writers prefer for the present to accept the Ehrlich mechanism, with the modifi-

cations suggested by Neubauer and Fromherz, as the source of fusel oil precursors, and the idea that the final steps are mediated by the carboxylase and hydrogen transfer systems of the zymase complex.

References

1. EHRLICH, F. *Ber.*, **40**, 1027 (1907).
2. NEUBAUER, O., and FROMHERZ, K. *Z. physiol. Chem.*, **70**, 326 (1911).
3. THORNE, R. S. W. *J. Inst. Brewing*, **55**, (N. S. 46), 201 (1949).
4. NEUBERG, C., and HILDESHEIMER, A. *Biochem. Z.*, **31**, 170 (1911).
5. EHRLICH, F. *Ber.*, **39**, 4072 (1906).
6. BUCHNER, E., and MEISENHEIMER, J. *Ibid.*, 3201.
7. PENNIMAN, W. B. D., SMITH, D. C., and LAWSHE, E. F. *Ind. Eng. Chem., Anal. Ed.*, **9**, 91 (1937).
8. *Methods of Analysis of the Association of Official Agricultural Chemists*, 6th ed. Washington, D. C.: AOAC (1945).
9. WEBB, A. D., and KEPNER, R. E. Personal communication (1951).
10. EHRLICH, F. *Biochem. Z.*, **2**, 52 (1906).
11. HARDEN, A. *Alcoholic Fermentation*, 4th ed. New York: Longmans, Green (1932).

Manuscript received July 7, 1951.

Prevention of Postharvest Decay of Stone Fruits by Volatile Chemicals

J. S. Vandemark and E. G. Sharvelle

*Departments of Horticulture and Botany
and Plant Pathology,
Purdue University, Lafayette, Indiana*

In-transit loss of peaches from disease is the limiting factor in the marketing of this crop. In spite of recommended field practices, brown rot losses in transit caused by *Monilinia fructicola* (Wint.) Honey¹ frequently exceed 10% of the crop value and greatly limit distribution.

In 1950, preliminary experiments were undertaken to investigate the possibility of using volatile chemicals to reduce the losses. Peaches and plums were inoculated artificially with a mixed spore suspension of *M. fructicola* and *Rhizopus* spp. These fruits were then incubated at 80° F and above 90% relative humidity for a 24-hr period prior to treatment with volatile chemicals. Replicated fruit samples were then placed in closed glass cylinders of 40-l capacity, inside of which the chemicals under test were introduced in open glass containers, permitting complete volatilization within the closed treatment chamber. After a 24-hr treatment period, the fruits were removed from the chemical atmosphere and were stored at laboratory temperature at a relatively high humidity for an additional period of 72 hr.

Trichloroethylene prevented all breakdown of the inoculated fruit and was effective at a concentration as low as 1:10,000 (1 vol nonvolatilized chemical in 10,000 vol atmos). No injury resulted to peaches and plums when exposed for 24 hr to concentrations of 1:4,000 and 1:10,000 of the chemical. Untreated con-

¹ In a recent publication (*Mycologia*, **37**, 648 [1945]), Whetzel recognizes the genus *Monillinia* Honey and thus treats this species as *Monillinia fructicola* (Wint.) Honey.

trol showed complete breakdown within this 5-day period.

Tetrachloroethylene and 1,1,2-trichloroethane also inhibited rot completely but caused some browning of the fruit at a concentration of 1:4,000 and less browning at 1:10,000. Observations suggest that much of this browning could be eliminated by circulating the atmosphere during the period of treatment, as the vapors apparently tend to stratify and settle to the bottom of the treatment containers.

S-tetrachloroethane inhibited all rot development completely at concentrations of 1:4,000 and 1:10,000 but also caused browning of the fruit. There is a possibility that lower concentrations of this chemical might be effective, since laboratory tests *in vitro* indicated that it will inhibit pure cultures of *Rhizopus* spp. and *M. fructicola* at concentrations as low as 1:20,000.

The above findings have been substantiated by pure culture toxicity tests in the laboratory with fungi as well as bacteria and are preliminary to more extensive studies now in progress on the evaluation of volatile chemicals as preventatives for market, storage, and in-transit losses to fruits and vegetables caused by microorganisms.

Manuscript received September 12, 1951.

Hemagglutinins in Caterpillar Bloods

Alan W. Bernheimer

The Biological Laboratory of the Long Island Biological Association, Cold Spring Harbor, New York, and the Department of Microbiology, New York University College of Medicine, New York

Although hemagglutinins are known to be present in a wide variety of animals and plants, their occurrence in insects does not appear to have been systematically investigated. During the course of experiments on the *Cecropia* moth, it was observed that the hemolymph of the larva and pupa of this species exhibits a relatively powerful agglutinating action when mixed with washed mammalian erythrocytes. The agglutinin was found to be present in titers of 1:25 to 1:625 in a large number of larvae and pupae examined, and appeared to be absent from the adult moth and from the egg.

The *cecropial* agglutinin is a labile substance, its titer being reduced by approximately one half upon heating the hemolymph, at pH 7.0, to 45° C for 1 hr, and by more than 97% at 55° C for 1 hr. It is largely or completely nondialyzable. No hemolysis occurred upon addition of guinea pig complement to mixtures of hemolymph and washed human erythrocytes.

It was of interest to examine other kinds of caterpillars for the presence or absence of hemagglutinin. To this end, specimens of hemolymph obtained from a total of 46 species of lepidopteran larvae,¹ represent-

¹ We are greatly indebted to James King, The Biological Laboratory, Cold Spring Harbor, N. Y., for aid in identification of many of the larvae, to Pauline James and Patricia Moore for help in collecting, and to Alexander S. Wiener for supplying some of the human blood specimens.

ing 16 families, were tested for ability to agglutinate human erythrocytes. One one-hundredth ml of hemolymph was mixed with 0.04 ml 3% suspension of washed human erythrocytes obtained from a Group O donor. The mixtures were made in transparent glass spot plates, incubated at room temperature, and read at intervals up to 60 min.

The results (Table 1) show that hemagglutinins are present in 10 of the 46 species examined. All the species showing agglutinating action were moth larvae; all the butterfly larvae were negative. Beyond this, however, little correlation between presence of

TABLE 1
PRESENCE OR ABSENCE OF HEMAGGLUTININ IN
HEMOLYMPH OF LEPIDOPTERAN LARVAE*

Heterocera		
<i>Protoparce sexta</i>	(8)†	0 to ++
<i>Ceratomia undulosa</i>	(1)	++
<i>Cressonia juglandis</i>	(5)	0
<i>Callosamia promethea</i>	(2)	0
<i>Samia cecropia</i>	(19)	+++
<i>Actias luna</i>	(3)	+ to +++
<i>Telea polyphemus</i>	(3)	0
<i>Automeris io</i>	(7)	0
<i>Citheronia regalis</i>	(8)	0
<i>Dryocampa rubicunda</i>	(4)	0
<i>Anisota senatoria</i>	(2)	0
Unidentified arctiid	(2)	0
" "	(1)	0
<i>Euchaetias egle</i>	(3)	0
<i>Isia isabella</i>	(1)	0
<i>Halisdota tessellaris</i>	(8)	+ to +++
<i>Halisdota caryae</i>	(9)	+++
<i>Acronycta americana</i>	(3)	0
<i>Acronycta</i> sp.	(1)	0
Unidentified noctuid	(1)	0
" "	(1)	0
" "	(1)	0
<i>Symmerista albifrons</i>	(3)	0
<i>Datana ministra</i>	(2)	0
<i>Datana ?integerima</i>	(8)‡	0 to ++
<i>Datana</i> sp.	(3)	0
<i>Hemerocampa leucostigma</i>	(3)	0
<i>Porthetria dispar</i>	(2)	0
<i>Malacosoma</i> sp?	(2)	0
<i>Sibine stimulae</i>	(4)	+++
<i>Prolimacodes badia</i>	(2)	0
<i>Phobetron pithecium</i>	(1)	0
Unidentified cochlidiid	(1)	0
<i>Galleria mellonella</i>	(4)	0
<i>Ephestia kuehniella</i>	(1)	0
Unidentified cossid	(1)	+
" tortricid	(1)	0
" "	(1)	+++
Rhopalocera		
<i>Danaus plexippus</i>	(3)	0
<i>Polyommata interrogationis</i>	(2)	0
<i>Aglais antiopa</i>	(2)	0
<i>Papilio troilus</i>	(3)	0
<i>Papilio turnus</i>	(1)	0
<i>Papilio polyxenes</i>	(1)	0
<i>Pieris rapae</i>	(1)	0
<i>Epargyreus tityrus</i>	(3)	0

* Figures indicate number of specimens of pools examined. Zero indicates no agglutination; +, barely visible agglutination; ++, erythrocytes in a great many small clumps; +++, a small number of large clumps; +++, a single large clump.

† Seven specimens positive and 1 negative.

‡ Five specimens positive and 3 negative.