of chromosomes in *Sorbus Aria*. Counts have not been obtained for *Aronia melanocarpa* but in view of the chromosome number and behavior in the hybrid both parents evidently have 17 gametic chromosomes which are completely compatible in the F_1 hybrid.

Sorbopyrus auricularis bulbiformis in the Arboretum is from scions sent from the Kew Gardens in England. This variety of *Sorbopyrus* is a seedling of *auricularis* and is more like the pear in fruit characters than the parental species.[3] A cytological study of the pollen mother cells shows that there are 17 paired and 17 single chromosomes at the first reduction division. Numerous counts show a total of 34 chromosomes at metaphase. In later stages of division 17 chromosomes can be counted at each pole and 17 dividing univalents between. The second division is also irregular and practically all of the pollen grains are aborted. *Sorbus Aria* has 17 gametic chromosomes and the species of *Pyrus* investigated by Dr. Nebel[1] also have 17 pairs of chromosomes. This variety of *Sorbopyrus* is evidently a back cross of a diploid egg cell of the F_1 hybrid with a haploid pollen grain from *Pyrus*. The presence of two sets of *Pyrus* chromosomes and one set of *Sorbus* chromosomes would explain why the variety is more like *Pyrus* than the parental hybrid. Evidently the *Sorbus* chromosomes will function in somatic development with either one or two sets of *Pyrus* chromosomes although the *Pyrus* and *Sorbus* chromosomes do not pair at the reduction divisions.

The chromosome behavior in these generic hybrids suggests that it may be necessary to make a taxonomic revision of the genera in the Pomoideae subfamily of the Rosaceae.

[1] Nebel, B., Zur Cytologie von Malus und Vitis. "Die Gartenbauwissenschaft," Band 1, *Heft.*, 6, 449–592 (1929).

[2] Rehder, A., *Manual of Cultivated Trees and Shrubs*, The McMillan Co., New York, 1927.

[3] Tatar, M. "Pirus Bollwylleriana De. C. var. bulbiformis," *Wien. Obst-ud Gart. Zeitung.*, 3, 26–28 (1878).

CHEMICAL ASPECTS OF DISEASE RESISTANCE IN THE ONION

BY J. C. WALKER, KARL PAUL LINK AND H. R. ANGELL

UNIVERSITY OF WISCONSIN AND OFFICE OF HORTICULTURAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE

Read before the Academy, April 23, 1929

Striking differences in susceptibility to one or another parasite are commonly noted among closely related horticultural varieties of our economic plants. Inquiry into the causes of disease resistance is fundamental to permanent progress in the improvement of cultivated crop

plants and offers an important present day challenge to pathologist, geneticist and biochemist.

In a previous communication[1] the senior writer called attention to the striking degree of resistance in colored varieties of onion to the disease known as smudge. This is a disease which appears on the bulb, and the neck tissue just above the latter, shortly before maturity. The causal fungus, *Colletotrichum circinans* (Berk.) Vogl., persists from year to year in the soil, and infects the outer dry scales of the susceptible varieties during the last three or four weeks of the growing season. At harvest it is evident as black smudgy spots on these outer scales. Following harvest the fungus penetrates the cuticle of the underlying living fleshy scales and causes a gradual shrinkage and decay during curing and storage.

In the colored varieties exposed to the parasite in a similar manner, very little or no infection of the dry outer scales occurs, the only conspicuous symptoms being on the outer sheaths of the leaves above the bulb where comparatively little pigment develops. This last type of infection is of little economic consequence since the infected parts are largely removed at harvest. There is a close correlation of the absence of infection in the resistant varieties with the occurrence of red or yellow outer scale tissue. Where such pigmentation is poorly developed, as in the neck tissue above the bulbs or where the pigment has become bleached by unusual exposure to rainy weather, infection does occur, but as a rule further ingress of the parasite is checked by the next underlying tissue which is normally colored.

As pointed out in earlier papers,[1] the crude water extract from dry outer colored scales (yellow or red) is distinctly toxic to the spores and thalli of the fungus. This is readily demonstrated by placing either in a moderate concentration of the extract. Growth of thalli is immediately checked if not completely inhibited. The germinating spore shows an unusual response. The germ tube starts off in the regular manner but within a very short time the growing tips either dissolve or rupture, the exact process not having been determined as yet. The protoplasm of the spore is forced out through the small opening and it congregates characteristically as a naked mass alongside the exterior of the spore. In this way the spore is rendered functionless. In cold water extracts from outer scales of white varieties, on the other hand, normal germ tubes develop, the attachment bodies (appressoria) form, and from the latter infection tubes arise.

The basic reason for the marked resistance of colored bulbs to this disease seems to be due to the fact that in the scales of such bulbs there is formed a substance (or substances) not present in the scales of white bulbs, which is toxic to the fungus. Being soluble in cold water it diffuses out readily from the dead cells of the outer scales into drops or films of

meteoric or soil water in contact with the bulbs. It thus retards or kills the fungus within such drops of water before it can establish itself upon the host tissue.

The relatively simple manner in which the resistant quality of colored bulbs was brought into effect suggested the possibility of isolating the chemical compound or compounds responsible. At this point the junior authors joined in the investigation and undertook the major responsibility for the chemical study.

Regular qualitative tests with ferric chloride solution, dilute ammoniacal solution of potassium ferricyanide, methylene blue, iodine solution with ten per cent ammonia, and bromine water showed the presence of phenolic compounds in water extracts from red and from yellow scales, and their absence in extracts from white scales. This reaction of the colored extracts was to be expected because the pigment substances themselves are supposedly phenolic derivatives and those soluble in water would thus give a positive test. The yellow pigment, quercetin, which exists in the scales in the free state, is practically insoluble in cold water. Thus the reactions indicated that other phenolic compounds, more readily soluble in water, exist in red and yellow scales. On the other hand, even phenolic compounds that give a positive reaction with ferric chloride seem to be absent in white scales. The possibility of glucosides of quercetin or of tannin compounds being present was considered but all attempts to demonstrate them yielded negative results.[2]

Assuming that the toxic compounds were phenolic in character, the next procedure was to attempt their isolation without destruction of the toxic properties. Neutral solvents were used at temperatures below 60° C. Acetone was found to be the most efficient solvent. Pigmented scales were subjected to an exhaustive extraction with dry acetone. The acetone extract was concentrated to dryness under reduced pressure and the resulting residue of extracted matter taken up with water. This eliminated quercetin and also permitted a partial separation of inactive impurities. The water soluble portion produced the same effect upon the spores as did crude water extracts made directly from the scales. It was next concentrated under reduced pressure below 40°C. to a thick syrup and treated with a mixture consisting of about 20 times its volume of alcohol and 40 times its volume of ether. A reddish brown precipitate was filtered off, which when dissolved in water showed very little toxicity. However, when the alcohol-ether solution was concentrated to dryness and the resulting residue taken up in water and tested upon the spores it proved to be very toxic. These experiments indicated that the toxic principle might be separated in its pure form by using neutral solvents and temperatures below 60°C.

The next step was an attempt to secure a large quantity of the toxic

principle. A slightly different procedure was followed to effect this objective. About fifteen pounds, amounting to several bushels, of dry outer scales of yellow onions were steeped in water overnight. The amount of free quercetin thus removed was negligible. The colored aqueous extract was treated first with neutral, then with basic lead actates until no more precipitate was obtained. The latter was filtered off, suspended in a large volume of water and hydrogen sulphide passed in to decompose the lead salt. The lead sulphide was filtered off leaving the toxic substance in solution together with impurities. This was concentrated under reduced pressure at 40–50° until the syrup contained 80 to 85 per cent of solids. After standing for 12 hours a dark brown gum separated leaving a thinner, aqueous syrup which gave a strong positive test with a solution of ferric chloride. From this syrup the major portion of the toxic principle was removed by adding successive portions of cold 95 per cent alcohol and quickly removing the alcoholic liquor before the gummy precipitate went into solution.

The alcoholic extracts thus obtained were highly pigmented, usually a deep red. Most of this color-producing principle was removed by heating the alcoholic extract under a reflux condenser in the presence of successive small portions of activated blood charcoal. After three such treatments the alcoholic solution attained a light brown color and still gave a strong color reaction with a ferric chloride solution, which indicated that the active substance, or substances, were still present. The alcohol was removed under reduced pressure at 35°. The yellow syrup obtained was taken up in warm 20 per cent alcohol, and warm petrol ether, b. p. 60–80° (1 part to 5) was added. The solution was then placed in a desiccator over calcium chloride. After three days, crystals formed on the sides of the vessel containing the solution. These crystals were removed, dried in a vacuum at 95°, and on purification were found to be pale yellow needles with a constant melting point of 199°. The substance was identified as the phenolic acid known as protocatechuic acid (3,4-dihydroxybenzoic acid), by preparing from it the ethyl and methyl esters and the diacetyl derivative, all of which exhibited melting points identical with the accepted constants for these substances. As a final criterion the ultimate analysis indicated that the isolated acid had the elementary composition of carbon and hydrogen in agreement with the theoretical values required by the formula $C_7H_6O_4$.[3]

The evidence points to the conclusion that this acid is one of the chief toxic substances which make up the disease-resistant principle of colored onion. It was prepared with equal facility from yellow and from red varieties and repeated attempts yielded none from the white susceptible varieties. Furthermore, at dilutions of 1–2000 to 1–3000 in distilled water it caused the typical rupturing of the spores of the parasite already de-

scribed as a characteristic property of the crude aqueous extract from colored scales. It should also be noted that the toxicity of the pure protocatechuic acid isolated from onion scales is identical with the toxicity of the pure acid from other sources, East Indian kino for instance. This fact serves as a criterion of biological purity and eliminates the possibility of the protocatechuic acid isolated from the onion scales being contaminated with minute traces of another very potent toxic entity.

The phenolic acid, protocatechuic acid, is widely distributed in plants as a constituent of many aromatic compounds, in the catechol tannins, in numerous resins and wood gums, in lignified wood, and as a constituent of various flavone and anthocyan pigments. Its occurrence in the free state has, however, been reported in only a few cases. It is significant that the flavonol pigment quercetin has long since been recognized to be one of the pigments in the colored onion. Protocatechuic acid is a constituent of the quercetin molecule. On alkaline fusion, quercetin yields oxalic acid, phloroglucinol and protocatechuic acid. The close association of protocatechuic acid to the pigment quercetin deserves emphasis but it should be borne in mind that protocatechuic acid itself is not a pigment. The isolation of protocatechuic acid from pigmented onion scales represents to our knowledge the third instance that this acid has been found associated with the flavonol quercetin.

Since the isolation of the acid was performed by the application of chemical methods that exclude the possibility of the acid having arisen from quercetin by decomposition, it is definitely established that protocatechuic acid exists in the free state, and that it is one of the toxic principles involved in the resistance exhibited by the pigmented onion to the smudge disease.

It should be pointed out that the toxicity of the crude aqueous extract is greater than the toxic effects that could be ascribed to the amount of protocatechuic acid that was isolated from a given unit of toxic extract. It is, therefore, possible that additional toxic substances are present or that all of the acid was not isolated by the methods employed. The investigations are being extended, therefore, with the purpose of isolating other toxic substances and if possible to increase the yield of protocatechuic acid.

To our knowledge this is the first time that disease resistance in plants has been attributed to a definite chemical entity present in the resistant host (the pigmented onions) and absent in the non-resistant host (the white onions).

[1] See J. C. Walker, "Studies on Disease Resistance in the Onion," *Proc. Nat. Acad. Sci.*, **11**, 183–189 (1925); also "Disease Resistance to Onion Smudge," *J. Agr. Research*, **24**, 1019–1040 (1923).

[2] The statement previously made (*Proc. Nat. Acad. Sci.*, **11**, 188) that quercetin

exists in the onion combined with glucose is therefore erroneous. Perkin and Hummel (*J. Chem. Soc.*, **69**, 1295–1298) also state they failed to find a glucoside of quercetin in onion.

[3] For further details regarding the isolation and identification of the acid, see Link, Angell and Walker, "The Isolation of Protocatechuic Acid from Pigmented Onion Scales and Its Significance in Relation to Disease Resistance in Onions," *J. Biol. Chem.*, **81**, 369–375 (1929); and Angell, Walker and Link, "A Chemical Compound Responsible for Disease Resistance in the Onion," *J. Agr. Research* (1929). In press.

ON THE EXISTENCE OF INTEGRALS OF THE SYSTEM OF PARTIAL DIFFERENTIAL EQUATIONS $A^i_{\alpha\beta i} = 0$ *IN n VARIABLES*

By Tracy Yerkes Thomas

Department of Mathematics, Princeton University

Communicated October 14, 1929

§1. In this note we consider the integrals $\Gamma^i_{\alpha\beta}(=\Gamma^i_{\alpha\beta})$ of the system of equations

$$A^i_{\alpha\beta i} = 0 \qquad \text{(a)}$$

where the quantities $A^i_{\alpha\beta\gamma}$ are the components of the affine normal tensor.[1] Since the components $A^i_{\alpha\beta\gamma}$ are definite linear expressions in the components of affine connection $\Gamma^i_{\alpha\beta}(x)$ and their first derivatives, equations (a) constitute a system of linear partial differential equations of the first order in a set of unknown functions $\Gamma^i_{\alpha\beta}$. These equations are, in fact, equivalent to the system of equations obtained by setting the contracted affine curvature tensor $\beta^i_{\alpha\beta i}$ equal to zero, i.e.,

$$\frac{\partial\Gamma^i_{\alpha\beta}}{\partial x^i} - \frac{\partial\Gamma^i_{\alpha i}}{\partial x\beta} + \Gamma^\sigma_{\alpha\beta}\Gamma^i_{\sigma i} - \Gamma^\sigma_{\alpha i}\Gamma^i_{\sigma\beta} = 0.$$

We use the normal tensor $A^i_{\alpha\beta\gamma}$ instead of the equivalent curvature tensor $\beta^i_{\alpha\beta\gamma}$ as the former is more readily adapted to most theoretical investigations. When reference is made to a system of normal coördinates y^i instead of to arbitrary coördinates x^i, the designation $C^i_{\alpha\beta}(y)$ instead of $\Gamma^i_{\alpha\beta}(x)$ will be used. We first treat the case $n = 2$ for which the existence of integrals $\Gamma^i_{\alpha\beta}$ or $C^i_{\alpha\beta}$ of the system (a) is immediate when use is made of one of the results of an earlier article (§3).[2] The general case $n \geqq 3$ is then treated (§4) and a corresponding existence theorem is established. We have not given the convergence proof for the formal power series expansions of the components $A^i_{\alpha\beta\gamma}$ for $n \geqq 3$ as it is intended to publish this later as part of a systematic account of the existence of integrals of systems of tensor equations of the above type (a).