Resistance of Apple to Powdery Mildew and Its Relationship to Osmotic Activity of the Cell Sap

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ABSTRACT

High osmotic values of cell sap are associated with resistance of apple to powdery mildew. This relationship occurs within a single variety in comparisons between young susceptible and old resistant leaves and among four clones of McIntosh having different levels of resistance. Other unidentified physiological factors appear to contribute to the resistance mechanism. Phytopathology 60:1848-1849.

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Powdery mildew of apply, *Malus* spp., incited by the fungus, *Podosphaera leucotricha* (Ell. & Ev.) Salm., is a serious disease in many fruit-growing areas. Etiology of the disease was described by Woodward (14) in 1927, and the present host range includes most of the *Malus* species (12). In 1949, Dickinson (1) noted that the spore tubes of *P. leucotricha* grew away from wet surfaces, and that contact with a membrane or cell wall stimulated appressorial development, with subsequent growth limited by the formation of haustoria (2).

Very little information is available on the mechanism of host resistance. Conidia of *P. leucotricha* germinate and develop extensive germ tube growth on oak leaves, but fail to penetrate the tissue (14). This suggests that morphological characters of the host prevent penetration by the fungus and further development of the disease. Internal factors also may be involved in host resistance to *P. leucotricha.* This has been shown in *Prunus* by Grainger (5), who listed data demonstrating an increase in the reducing sugars of leaf tissue after infection with *P. oxyacanthae.* Other workers (4, 13) have found an association between high osmotic values and resistance to mildew in grape and peach.

Inheritance of resistance to powdery mildew in apple has been shown to be monogenic by Mowry (9). Earlier work was complicated by factors introduced by aging or because workers used different varieties. The availability of McIntosh clones with different degrees of resistance produced by ionizing radiation (8) permitted us to examine clones of the same cultivar.

Four 1-year-old nursery trees of each clone of McIntosh apple were selected for uniformity, potted in No. 10 cans with standard greenhouse soil mix, and grown for 1 growing season in a location free from mildew. After the completion of the dormancy period, they were used for host-parasite studies.

Five fully differentiated young leaves of the same physiological age from each clone were placed in petri dishes containing moist filter paper. Controls consisted of clean glass slides placed in similar petri dishes. Leaves and slides were inoculated with conidia of *P. leucotricha,* using a method similar to that of Wartenberg (12), and the number of colonies on the leaves was counted after 6 days' incubation at 22 C. Thickness of epidermal cell walls was measured by an optical micrometer on thinly sectioned leaf tissues. Resistance to penetration was determined with a Precision Penetrometer (Precision Scientific Co., Chicago, Ill.) equipped with a special 0.5-mm needle by the method of Tressler et al. (11). Leaf sap was extracted from frozen leaves by means of a French press. The resulting decoction was centrifuged at 15,000 *g,* the supernatant diluted 50% with glass distilled water, and the osmotic value determined by the depression of the freezing point (6). Similar studies were made by comparing young susceptible leaves with older resistant leaves from individual trees of different cultivars of known field susceptibility. These data were subjected to the analysis of variance (10) and Duncan's multiple range test (3) for statistical significance.

Differences in mildew susceptibility of detached leaves from the McIntosh clones indicated that C-10, with an average of 4.6 colonies on 30 detached leaves, was the most resistant, while the local clone, C-106, with 24.5 colonies, was the most susceptible. Clones C-17 and C-41 were intermediate. Direct comparisons made on detached leaves of C-10 and C-106 showed no differences in percentage of germination, but germ tube length on the epidermis of C-10 averaged 85.2 μ and was 70% greater than that found on the C-10. There was an earlier development of conidiophores and appressoria on C-106. Growth of *P. leucotricha* on detached leaves without their epidermis was depressed by C-10 and stimulated by C-106, indicating that internal factors play an important role in the growth of *P. leucotricha* and may be related to resistance.

Morphological differences among the 4 clones (Table 1) showed that clone C-10 had the greatest resistance to penetration, and C-41 the least, the others being intermediate. Since C-17 had a thinner wall than C-41 and there were no differences between C-10 and C-106, thickness of the cell wall does not appear to be associated with resistance to penetration. Osmotic value difference indicates that low osmotic value was associated with susceptibility, as C-106 had the lowest value and C-10 the highest, while C-41 and C-17 were intermediate. Other observations included comparisons of young and old leaves of Cortland, Jonathan, and McIntosh (clone C-NF), and demonstrate (Table 2) that the cell wall thickness increases with age in all varieties but the osmotic values increase only in the McIntosh and Jonathan clones.

Both external and internal factors apparently affect the resistance of apple to infection by *P. leucotricha.* No correlations between resistance and thickness of the

TABLE 1. Thickness of epidermal cell wall, resistance to mechanical penetration, and osmotic values of expressed sap of leaves of four McIntosh apple clones with varying degrees of susceptibility to *Podosphaera leucotricha*[a]

Clone	Reaction	Thickness of[b] cell wall, μ	Depth of[c] penetration, μ	Osmotic value[d] of expressed sap
C-106	VS[e]	1.70 a	700 a	9.2 a
C-41	VS	1.19 b	800 b	16.4 b
C-17	S	1.04 c	660 a	14.2 c
C-10	R	1.63 a	600 c	20.6 d

[a] Means within a horizontal column followed by the same letter(s) are not significantly different at the .05 probability level.
[b] Average of 100 measurements.
[c] Average of 30 determinations.
[d] Average of 3 measurements; expressed as atmospheres.
[e] VS = Very susceptible, S = Susceptible, R = Resistant.

TABLE 2. Reaction to *Podosphaera leucotricha*, epidermal cell wall thickness and osmotic values of expressed sap of leaves of different ages from Cortland, Jonathan, and clone NF[a]

Variety	Age of leaf	Mildew infection rating	Thickness of[b] cell wall, μ	Osmotic value[c] of expressed sap
Cortland	Old	S-R[e]	2.87 a	16.6 ab
	Young	VS	2.06 b	15.0 a
Jonathan	Old	R	3.03 c	20.0 c
	Young	VS	1.69 d	15.9 a
McIntosh NF	Old	R	2.48 e	19.1 c
	Young	S	1.70 d	17.1 a
Peach[d]	Old	R		20.7
	Young	VS		16.8

[a] Means within a horizontal column followed by the same letter(s) are not significantly different at the .05 probability level.
[b] Average of 100 measurements.
[c] Average of 3 determinations.
[d] Data from Weinhold & English (13).
[e] VS = Very susceptible, S = Susceptible, R = Resistant.

cell wall were found among the McIntosh clones, but a greater resistance to mechanical penetration and decreased appressoria formation are characteristic of resistant clone, C-10. These observations suggest that the cuticle may provide a mechanical barrier and/or possess substances which inhibit penetration. Other resistant varieties of apple contain fungistatic substances in their cuticle (7) which are active in inhibiting germination, but other substances also may be present which inhibit appressorial development.

Internal factors appear to be more important deterrents to infection by *P. leucotricha* than mechanical ones. The decreased conidial germ tube growth on leaves of C-10 stripped of their epidermis suggested that resistant leaves may contain substances inhibitory to the fungus. Such substances may depress growth because of their toxic nature, or by creating an unfavorable environment for growth through increased osmotic values.

McIntosh C-10 had an osmotic value of 20.6 atm, twice that of the susceptible C-106. These data agree with observations in peach (13) that mildew resistance is accompanied by an increase in osmotic values from 16.8 to 20.7 atm. The osmotic values of susceptible and resistant leaves of Jonathan and clone C-NF also compare favorably with the peach measurements. Both young and old leaves of Cortland gave values indicating susceptibility. Cortland, however, is our most susceptible variety, and its mature leaves do not possess a high level of resistance. We now have an irradiated clone of Cortland with enhanced resistance (8), and can re-examine this point.

These observations provide additional evidence that increasing osmotic values are associated with increasing mildew resistance. This was demonstrated in clones of the same variety as well as in young and old leaves of unrelated varieties. Other unidentified substances in the leaf tissue of some clones appear to contribute to this resistance phenomena by restricting conidia germ tube growth in leaves stripped of their epidermis.

LITERATURE CITED

1. DICKINSON, S. 1949. Studies in the physiology of obligate parasitism. I. The stimuli determining the direction of growth of the germ tubes of rust and mildew spores. Ann. Appl. Biol. 13:89-104.
2. DICKINSON, S. 1949. Studies in the physiology of obligate parasitism. IV. The formation on membranes of haustoria by rust hypha and powdery mildew germ tubes. Ann. Appl. Biol. 13:344-353.
3. DUNCAN, D. B. 1955. New multiple range and multiple F tests. Biometrics 11:1-42.
4. GOHEEN, A. C., & W. C. SCHNATHORST. 1963. Resistance to powdery mildew in grapevines related to osmotic value and associated factors. Phytopathology 53:1139 (Abstr.).
5. GRAINGER, J. 1947. The ecology of *Erysiphe graminis*. Brit. Mycol. Soc. Trans. 31:54-65.
6. HARRIS, J. S., & R. A. GORTNER. 1914. Notes on the calculation of the osmotic pressure of expressed vegetable saps for the depression of freezing point, with a table for the values of P for $\Delta = 0.0001$ to $\Delta = 2.999$. Amer. J. Bot. 1:75-78.
7. MARTIN, J. T., F. R. BATT, & R. T. BURCHILL. 1957. Fungistatic properties of apple leaf wax. Nature 180: 796-797.
8. MCINTOSH, D. L., & K. LAPINS. 1965. Differences in susceptibility of apple powdery mildew observed in McIntosh clones after exposure to ionizing radiation. Can. J. Plant Sci. 46:619-623.
9. MOWRY, J. B. 1965. Inheritance of susceptibility of apple to *Podosphaera leucotricha*. Phytopathology 55:76-78.
10. SNEDECOR, G. W., & W. G. COCHRAN. 1967. Statistical methods. [6th ed.] Iowa State Univ. Press. p. 539.
11. TRESSLER, D. K., C. BIRDSEYE, & W. T. MARRAY. 1932. Tenderness of meat. I. Determination of relative tenderness of chilled and quick frozen beef. Ind. Eng. Chem. 24:242-245.
12. WARTENBERG, W. 1960. Studien am Apfelmehltau, *Podosphaera leucotricha* (Ell. and Ev.) Salm. mit Berucksichtingung einer normergisch-plasmatachen Absehrreaktion. Phytopathol. Z. 39:16-64.
13. WEINHOLD, A. R., & H. ENGLISH. 1964. Significance of morphological barriers and osmotic pressure in resistance of mature peach leaves to powdery mildew. Phytopathology 54:1409-1414.
14. WOODWARD, R. C. 1927. Studies on *Podosphaera leucotricha* (Ell. & Ev.) Salm. I. Mode of penetration. Brit. Mycol. Soc. Trans. 12:173-204.