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Growth and Respiration of Dormant Flower Buds of *Pyrus communis* and *Pyrus calleryana*[1]

Mary E. Cole,[2] Theophanes Solomos,[3] and Miklos Faust[4]
Fruit Laboratory, Agricultural Research, U. S. Department of Agriculture, Beltsville, MD 20705

Additional index words. Prunus armeniaca, Prunus salicina, Prunus avium, Prunus domestica, Prunis serrulata, Malus hupehensis, Malus domestica

Abstract. **Respiration of flower-buds of *Pyrus communis* L., a late blooming species, and *P. calleryana*, an early blooming species, was investigated throughout the winter. Respiration of *P. calleryana* Decne at 5°C was twice as high as that of *P. communis*, whereas the respiration rates were similar at 25°. A large portion (60–70%) of the respiration at 5° was cyanide resistant in *P. calleryana* and much less in *P. communis*. The combination of inhibitors, cyanide (KCN) and salicylhydroxamic acid (SHAM), still only partially inhibited respiration. The residual respiration was much higher for *P. calleryana* than for *P. communis*. The nature of the residual respiration is not known.**

It appears that respiration of *P. calleryana* is less sensitive to cold than that of *P. communis*. *P. calleryana* also appears to complete the development of its buds during the winter and blooms early. In contrast, *P. communis*, the species which has a respiratory pathway less operational at relatively low temperatures, delays the completion of its buds until the spring when the temperature warms up, and consequently flowers later. This hypothesis was evaluated by measuring the mid-winter respiration of 5 early and 5 late blooming species of fruit trees, and the bloom date was evaluated relative to the type of respiration as described above.

Resting buds of fruit trees in general and pears in particular enter into dormancy with partially developed buds. The buds apparently complete their development by the time growth is resumed. The time of bloom varies from year to year and from species to species. There can be differences of up to one month for a species in a given location (2). The major factor regulating the time of bloom is temperature. A certain amount of cold is required to break the rest. After the cold requirement has been satisfied, a certain amount of heat is needed to enable the tree to bloom. It is conceivable that the time of bloom depends on the rate of development of buds during dormancy which in turn is influenced by temperature.

The time of bloom can be genetically transmitted (9) and intermediate bloom types created between early and late blooming pears. Generally the low chilling types (*P. calleryana*) also bloom early. The late blooming types (*P. communis*) bloom earlier when grafted on roots of early blooming types (*P. calleryana*) (24). Strausz (20) stated that this translocatable reduction of chilling requirement was due to a shift in growth promoters.

Pear buds are metabolically active during the winter. Thom (21) measured respiration of dormant buds of *P. communis* 'Hardy' and found that O_2 consumption increased six-fold when growth resumed in the spring. Zimmerman et al. (26) found that incorporation of uracil and valine into alcohol insoluble components of buds was high during the winter indicating that pear buds were not "resting" but metabolizing actively without visible in-

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[2]Present Address: Dept. of Agr. Economics, University of Maryland, College Park, MD, 20742.
[3]Professor, Dept. of Horticulture, University of Maryland, College Park, MD 20742.
[4]Plant Physiologist, Fruit Laboratory, SEA, U. S. Department of Agriculture, Beltsville, MD 20705.
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dication of growth. Chaudhry et al. (5) found that at the time of bud break concentrations of soluble-N, ester-P, and RNA-P increased markedly.

Strausz (20) found that *P. communis* had considerably higher levels of abscisic acid (ABA) than did *P. calleryana* during the winter. However, ABA did not disappear from the whole buds when rest was broken, nor did it disappear gradually during chilling. An unidentified growth promoter did not appear in significantly large amounts until growth had resumed.

Brown and Kotob (4) reported that the fresh and dry weights of *P. communis* flower buds slightly increased during October and November. Development of bud weight increases have not been reported for *P. calleryana* although the buds of *P. calleryana* are larger than those of *P. communis* at the end of the growing period.

None of the information available to date explains the major physiological differences in time of bloom between early and late blooming types. The purpose of this work was to examine the respiratory mechanism of *P. communis* and *P. calleryana* buds to discover the sources of energy for dormant bud development and find explanations for differences in bloom.

Materials and Methods

Plant material. Flower buds from two species of pear, *Pyrus communis* 'US 309' and *P. calleryana* 'Bradford' were used in the studies reported here. For comparative studies additional deciduous fruit tree species were included. These species were: *Malus hupehensis* (Pamp.) Rehd; *M. sylvestris* Mill; 'Golden Delicious'; *Prunus salicina* Lindl.; *P. domestica* L.; *P. avium* L.; *P. serrulata* Lindl.; 'Kwanzan'; *P. armeniaca* L.; 'Wilson Delicious'; and *P. armeniaca* L. 'US 566'.

Measurement of respiration. The scales were removed from the flower buds leaving the partially developed flower clusters (later referred to as inflorescences) intact. Enough inflorescences were prepared to obtain approximately 100 mg of tissue per flask. These were weighed and placed in Gilson respiration flasks. The concentrations of the reagents used were as follows: 3mM phosphate buffer, pH 5.3, 3mM SHAM and 0.2mM KCN. The method of Robbie (17) was used to absorb CO_2 and maintain constant cyanide concentration in the fluid. The rate of oxygen uptake is expressed as $\mu l\ 0_2$ at NTP/$g^{-1}h^{-1}$ (23).

The respiration rate of the pear flower bud inflorescences was measured at 25°, 18°, 10°, and 5°C periodically throughout dormancy. Since respiration at 18° and 10° was intermedite between the 25° and 5° data it is not reported here but is available elsewhere (6). The survey of respiration rates of the other species was performed in early February.

Ambient orchard temperatures. Chill units were calculated by summing the hours when the temperatures were between 7.2° and 0°C for each day. Heat sums, in degree-days above 4.5°, were calculated by summing the differences between 4.5° and the average daily temperatures after January 31.

Measurement of bud growth. Whole buds and inflorescences were weighed to determine bud growth each time buds were prepared for respiration.

Results

Respiration rates of Pyrus calleryana inflorescences. At 25°C the rate of 0_2 uptake of *P. calleryana* inflorescences increased steadily from late September until early March and then began to increase rapidly (Fig. 1). SHAM had no effect on the rate of respiration at 25° (Fig. 1B). The effect of cyanide on the rate of oxygen uptake varied with the calendar date. Thus, the inhibitor decreased respiration by about 50% from late September through the middle of October. This inhibition was decreased to about 20% in November, to gradually increase to 30–35% in late February and early March. It should be noted that the absolute values of the cyanide-resistant respiration followed the same pattern as the control samples up to early March. Thereafter, cyanide-resistant respiration declined sharply while that of the control increased rapidly. These changes in the absolute magnitudes of the two respiratory components resulted in respiration being strongly inhibited by cyanide in the latter part of March.

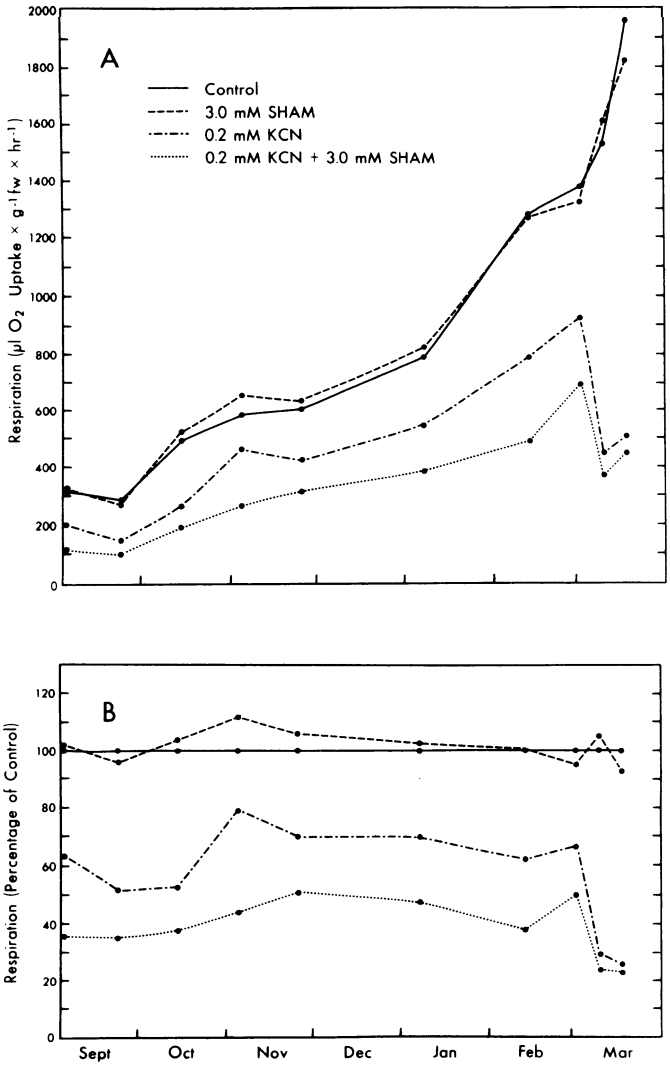

Fig. 1. Respiration of *Pyrus calleryana* inflorescences measured at 25°C.

In most plant tissues the combined application of KCN and SHAM does not inhibit completely the rate of oxygen uptake (7). The nature of this "residual" rate of respiration is unknown. Residual respiration increased slightly during the fall and winter, to decrease to almost zero in the latter part of March (Fig. 1A, B). At this stage, the inhibition of oxygen uptake by the combined application of SHAM and KCN was similar to that produced by cyanide alone.

At 5°C, respiration of the controls also increased steadily through the year (Fig. 2A). Further, the increment was similar to

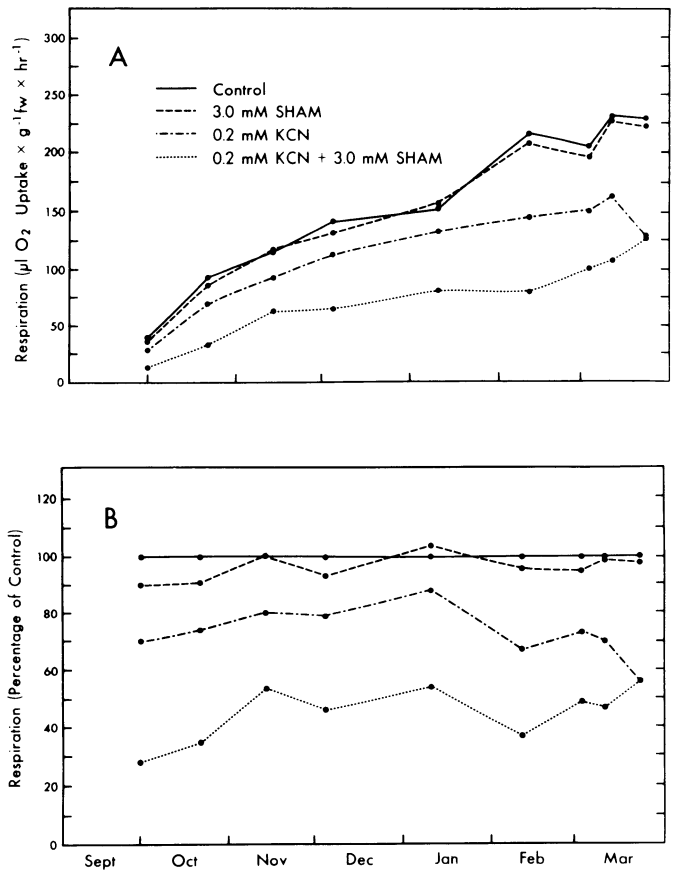

Fig. 2. Respiration of *Pyrus calleryana* inflorescences measured at 5°C.

that of 25°: about 600% (Fig. 2A). However, cyanide resistant respiration was higher at 5° that at 25°. In early October, cyanide reduced respiration by 30% while in January the inhibition was only 12%. In addition, not only did the percentage inhibition decrease during the fall and winter, but the absolute values of the cyanide-resistant component of respiration increased during the same period. In late March, cyanide inhibited respiration by 44%. Residual respiration increased during the period of rest, to become zero by late March (Fig. 2A, B).

Respiration of P. communis inflorescences. The respiration of *P. communis* inflorescences was also measured at 25° and 5°C from late fall until spring. As with *P. calleryana*, respiration of *P. communis* also increased during the above period. At 25° the respiration of the controls increased by about four-fold (Fig. 3A), cyanide inhibited respiration by 37% in late September and the inhibitory effect tended to increased with time (Fig. 3B), in contrast to the decrease with flower bud axes of *P. calleryana* (Fig. 1B). The residual respiration was also lower with *P. communis* than *P. calleryana*. The combined application of 3 mM SHAM and 0.2 mM KCN decreased respiration by 46% more than with cyanide alone in September (Fig. 3B). This difference decreased to only 10% from December through February. In early March it increased to 26%, to fall again to zero by the end of March (Fig. 3B).

The pattern of respiration at 5°C was similar to that at 25° (Figs. 4A, 3A). The inhibition by cyanide was lower at 5° that 25° (Fig. 4B). Thus, cyanide inhibited respiration by an average of 45% and 59% at 5° and 25°, respectively. Residual respiration was low at 5°. In September and October it averaged 30% below that caused by cyanide alone. The difference increased to 58% in November and then slowly decreased to 3% by late March.

Bud growth. The fresh weight of *P. calleryana* whole buds increased gradually from the fall unti mid-February and then began to increase sharply. With *P. communis*, whole flower buds doubled in weight during November after which bud weight remained constant from early December until late February when active growth began. The growth of the inflorescences followed a pattern almost identical to that of *P. calleryana*. Throughout the year the weight of the inflorescences of *P. calleryana* flower buds was a much smaller percentage of the total bud weight than for *P. communis* (Fig. 5).

Respiration of inflorescences of several species of fruit trees. As measured at 5°C the mid-winter respiration rates of inflorescences of the earlier blooming fruit trees were higher than those of the later blooming types, with the exception of *P. avium* (Table 1).

The average percentage of respiration resistant to cyanide was higher for the earlier blooming types than for the later blooming ones.

With the exception of *Prunus serrulata* the respiration, which was resistant to the combined treatment of SHAM and KCN, was higher for the earlier blooming types.

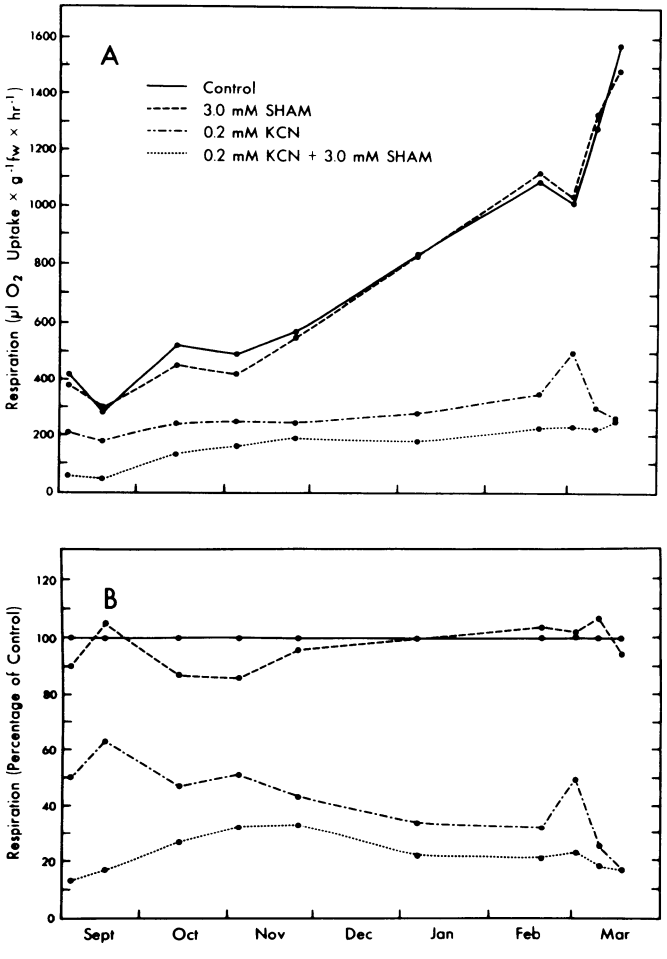

Fig. 3. Respiration of *Pyrus communis* inflorescences measured at 25°C.

Table 1. Mid-winter respiration rates of the flower buds of several species of deciduous fruit trees measured at 5° C.

Species	Date of full bloom	Inflorescences (g)	Respiration (μl/O_2 g^{-1} fresh wt) Control	3 mM SHAM	0.2 mM KCN	KCN + SHAM	%of control 3 mM SHAM	0.2 mM KCN	KCN+ SHAM
Prunus armeniaca 'Wilson Delicious'	Apr. 5	169	177	149	104	92	84	59	52
Prunus armeniaca 'US 566'	Apr. 8	137	193	177	154	122	92	80	63
Prunus salicina	Apr. 8	403	202	181	182	170	90	90	85
Pyrus calleryana	Apr.10	48	184	182	139	81	99	76	46
Prunus avium	Apr. 11	82	82	79	71	45	97	87	55
Pyrus communis	Apr. 17	88	111	114	51	14	103	46	13
Prunus domestica	Apr. 18	211	112	103	59	33	92	53	30
Malus hupenhensis	Apr. 18	133	165	163	85	56	99	52	34
Malus domestica 'Golden Delicious'	Apr. 24	48	81	85	63	31	104	77	38
Prunus serrulata	Apr. 26	105	87	98	75	55	113	86	63

Calculations using linear regression showed that there was no correlation between the number of inflorescences per gram and the rate of respiration.

Ambient orchard temperatures. Rest is assumed to be completed in pears after exposure to about 1,100 hours of chilling. This would have occurred about the second week of January in the Beltsville area (Fig. 6).

The heat sums required for bloom are shown in Figure 6. *P. calleryana* bloomed on April 10 after 180 degree-days of heat. *P. communis*, which flowered on April 17, required 233 degree-days.

Discussion

Since outdoor temperatures were low during dormancy, ranging from –10° to 10°C, the respiration at 5° may represent more closely what occurred in the buds in nature whereas the data obtained at 25° indicate the capacity of buds to respire. Both rates, the assumed natural respiration and the capacity of respiration were high for both species. Rates were in the range of 50 to 200 and 400 to 2000 μl 0_2 g^{-1} fw hr^{-1} for 5° and 25° respectively.

At 5°C the midwinter respiration of *P. calleryana* was double that of *P. communis*. In contrast, the difference in respiration be-

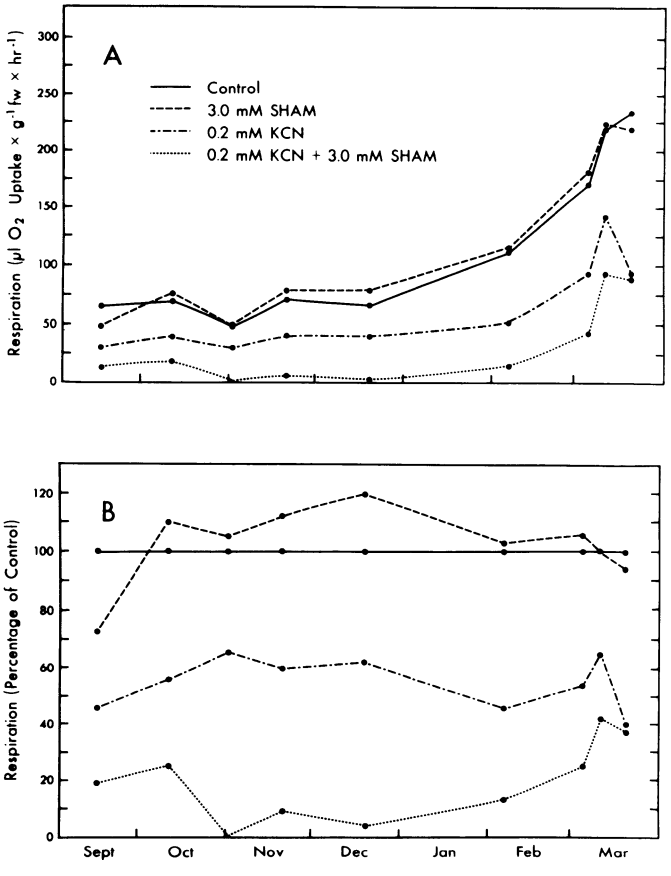

Fig. 4. Respiration of *Pyrus communis* inflorescences measured at 5°C.

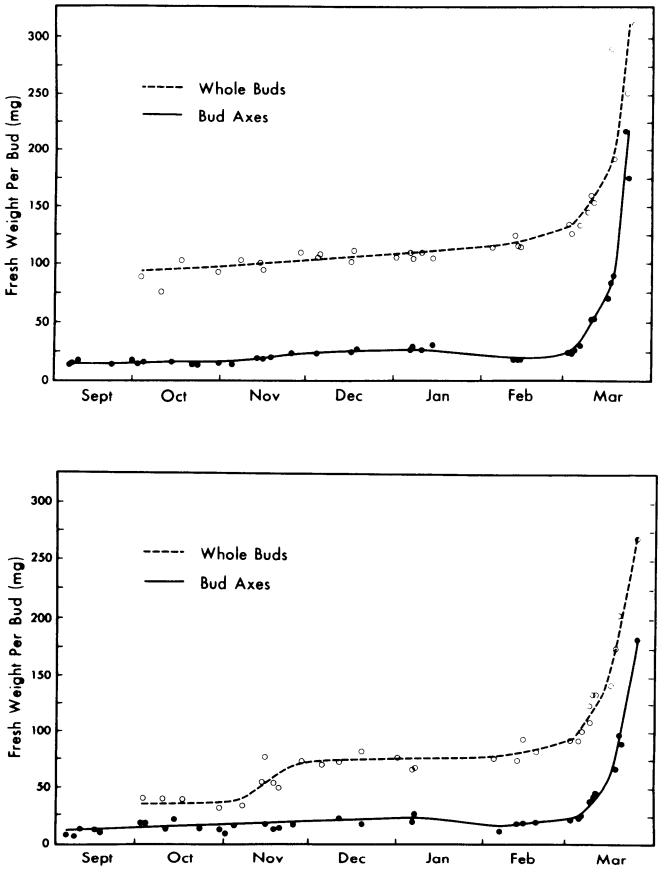

Fig. 5. Fresh weights of whole flower buds and inflorescences of *Pyrus calleryana* (upper) and *P. communis* (lower).

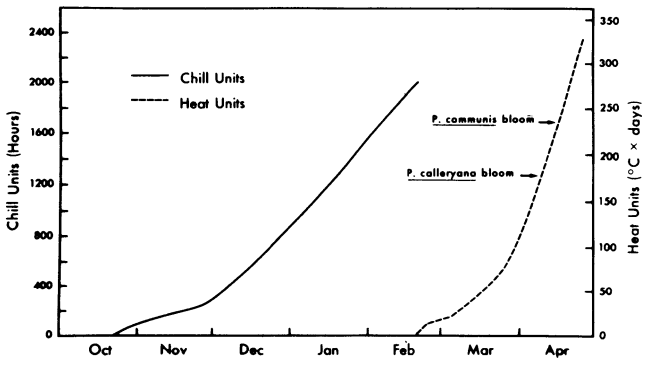

Fig. 6. Chill units (hours) and heat units (°C x days) for the 1979–1980 season.

tween the two species at 25° was much less indicating that *P. calleryana* has a mechanism which provides an advantage to this species at low temperature only. The largest increase in respiration of *P. calleryana* at 5° occurred during the month of January, whereas the increase of *P. communis* came a month later. At 25° both species have shown further elevation of respiration during the month of March. The increase in respiration during January and February may indicate the end of the rest period for each species, respectively. The increase in respiration during March indicated the beginning of spring metabolic activity even though actual flowering occurred later in mid-April. These results are consistent with those of Thom (21) who found that *P. communis* buds kept in cold storage at 2.5° increased their respiration continuously for five months. However, the pear data differ from the respiration of buds in warm climates which was shown by Lavee (14) to decrease when buds go into dormancy and increase when dormancy ends.

It is noteworthy that the dormant buds of pears respire at low temperatures (5°C) and the inhibitor experiments indicate that at this temperature the capacity for both alternate and residual oxidases is much larger than at 25°. Up to 80% of the respiration of *P. calleryana* buds was resistant to cyanide inhibition. The cyanide resistant respiration of *P. communis* buds was much lower. This is consistent with the result of other researchers who demonstrated that chilling can stimulate respiration in many species. Goodwine et al. (11) and Shulze et al. (18) showed that a temperature of 1° stimulates cyanide resistant respiration in potato tubers. Leopold and Musgrave (15) showed that chilling of soybean cotyledons engages the cyanide resistant alternate pathway, and Yoshida and Tagawa (25) found that most of the respiration of *Cornus* callus tissue was cyanide resistant below 15°.

Cyanide-resistant respiration is a widespread phenomenon in higher plants (7, 12, 19). It has been suggested by Bonner (3) that the cyanide-resistant pathway may be a general constituent of all plants with resistance varying from as low as a few percent (7, 12) to as much as 100 percent in *Arum maculatum* (13). On the basis of the present data, it is not clear whether cyanide-resistant respiration is engaged in the absence of cyanide. It should be noted, however, that both the absolute value of cyanide-resistant respiration and the percent inhibition decrease in late March, when the growth resumes, and hence the demand for energy increases.

The combination of both inhibitors, SHAM and KCN, still only partially inhibited respiration. The residual respiration was much higher for *P. calleryana* than for *P. communis*. The nature of the residual respiration is not known. Tucker (22) proposed that there may be a third pathway of respiration. Eskin et al. (8), Parrish and Leopold (16), and Goldstein et al. (10) all pointed out the possibility that lipoxygenase activity may be responsible for residual 0_2 uptake. Since SHAM appears to inhibit lipoxygenase activity (10) this possibility is unlikely in our experiments.

During these experiments, the picture which emerged was that the species (*P. calleryana*) having a higher rate of respiration, including the cyanide resistant alternate pathway and residual respiration, had the advantage of completing the development of its buds at low temperature and bloomed early. In contrast, the species which did not have a relatively active respiratory pathway operational at low temperatures, or had it to a lesser degree, needed to wait until completion of bud development took place during the spring when the temperature warmed up.

To check this theory we determined midwinter respiration of several early and late blooming species at 5°C. In general the survey of 10 species has shown that the above supposition was correct with one exception. *Prunus serrulata* bloomed late but its respiration was more like that of the early-blooming species.

The results presented here strongly suggest that buds, which respire through a pathway that is not inhibited, or is inhibited to a lesser degree, by cold, complete their development and bloom earlier than do buds which do not have this ability. Much of the development may occur in early spring. This study also revealed that before bloom the buds switch to the cyanide sensitive pathway which is also temperature sensitive. Thus, decreasing the temperature at this time appears to be an effective way to delay bloom. In fact this is what happened in apples when sprinkler irrigation was used for delaying bloom (1). Controlling temperature by sprinkling is effective but impractical. Further work is necessary to find chemical controls for the respiratory shift which could delay bloom effectively.

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Selection for Resistance in *Phaseolus vulgaris* L. to White Mold Disease Caused by *Sclerotinia sclerotiorum* (Lib.) de Bary

M. H. Dickson, J. E. Hunter, M. A. Boettger, and J. A. Cigna
Departments of Seed and Vegetable Sciences and Plant Pathology, New York State Agricultural Experiment Station, Geneva, NY 14456

Additional index words. juvenile stem test, blossom test, genetic resistance

Abstract. **Three populations of beans were screened for resistance to white mold using a mycelium/juvenile stem inoculation (JSI) method on 3-week-old plants and an ascospore/blossom test on plants in bloom was used to test resistance of survivors of the JSI test. There were few survivors in the JSI test: 0.8% in the 4-way cross of susceptible x susceptible, 2% in the intermediate x intermediate crosses and 3.8% in 10 *P. coccineus* lines with intermediate resistance. JSI tests of the progeny produced more survivors, 17% from the first 2 populations. There was good agreement between the JSI test on juvenile plants and the ascospore/blossom tests on blossoming plants respectively. The JSI test appears to be an efficient method with which to identify individual plants with moderate resistance.**

White mold disease of beans has been neglected by bean breeders until recently. In 1973 Adams et al. (2) identified some *Phaseolus coccineus* lines with resistance. Abawi et al. (1) reported resistance in *P. vulgaris*, derived crosses with *P. coccineus*. Most *P. vulgaris* accessions were relatively susceptible. In field trials *P. vulgaris* cultivars Anderson et al. (3), Coyne et al. (5) and Schwartz et al. (10) reported some resistance which could be attributed to avoidance due to plant growth habit in some cases and resistance in others.

Abawi et al. (1) used blooming plants that were sprayed with a suspension of ascospores. Hunter et al. (8) modified Abawi's ascospore-blossom inoculation (ABI) method (1). They also developed a second procedure, called limited term inoculation (8) which is referred to as juvenile stem inoculation (JSI) in this paper.

The high reliability of JSI and ABI (8) screening methods made possible the screening of many accessions of *Phaseolus* spp. (7), which led to the identification of a number of plant introductions with varying levels of resistance.

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