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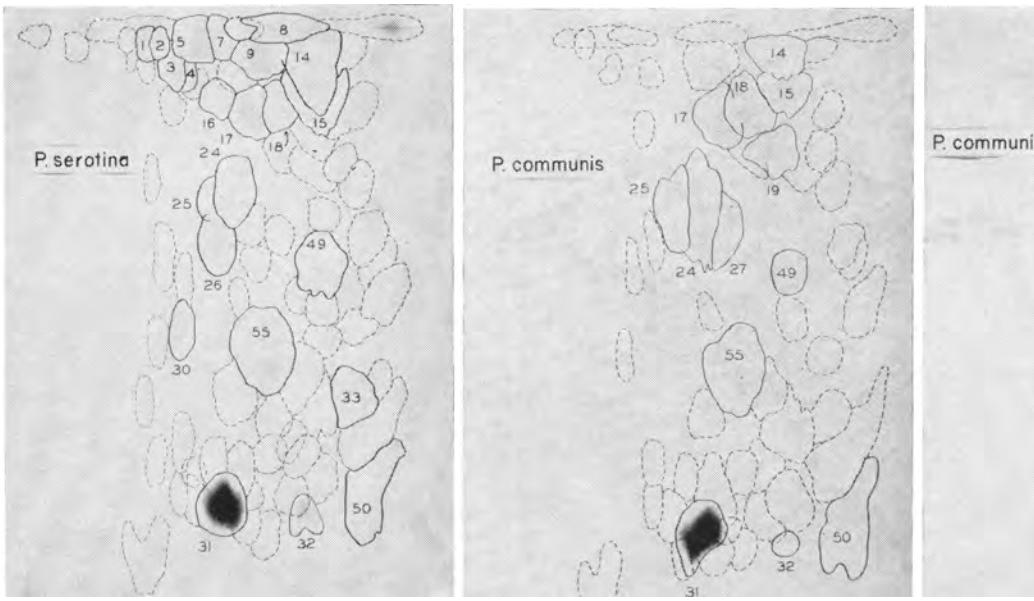


Paper chromatograms of leaf extracts of five pear species and one interspecific F_1 hybrid. All detectable spots—mostly polyphenolic compounds—are marked. Components used for identification are indicated by solid lines and are numbered. Spot size and, for some, color intensity are indicative of amounts present.

Chemical Identification of Pear Spec

THE OCCURRENCE of pear decline has varied with the species of rootstock used for commercial varieties of pear. Two species of oriental origin, *Pyrus serotina* and *P. ussuriensis*, are considered to be the most susceptible rootstocks. The degree of susceptibility of two other oriental species of rootstocks, *P. calleryana* and *P. betulaefolia*, is not well established. Although trees on *P. communis* rootstocks (European origin) are generally tolerant to decline, some losses have occurred.

Little accurate knowledge of rootstock identity exists in California where most trees are said to be planted on either *P. communis*—French, "domestic," seedlings of a commercial variety—or on "Japanese" rootstocks. The latter type implies *P. serotina* but also includes *P. ussuriensis*. Some *P. calleryana* and *P. betulaefolia* rootstocks have also been planted in California. Other oriental species as well as hybrids between *P. communis* and oriental species may also exist (see accompanying report).

With differential susceptibility a critical factor in pear decline it becomes important to know the identity of rootstocks for both diagnostic and experimental purposes. Identification of rootstocks based on vegetative characters of seedling rootsprouts is subject to question, however. Leaf morphology often varies greatly, even among leaves on a single shoot. Since leafy shoots from rootstocks are frequently absent in commercial orchards, identification techniques that would not require such material are desirable. However, *P. communis*, *P. serotina*, *P. ussuriensis*, and *P. calleryana* have been previously reported as not reliably separated by histological and morphological characteristics of roots.

The problem of pear species identification was investigated, therefore, by comparing polyphenolic compounds extracted from both leaves and bark of several species commonly used as rootstocks: *P. communis*, *P. serotina*, *P. ussuriensis*, *P. calleryana*, and *P. betulaefolia*.

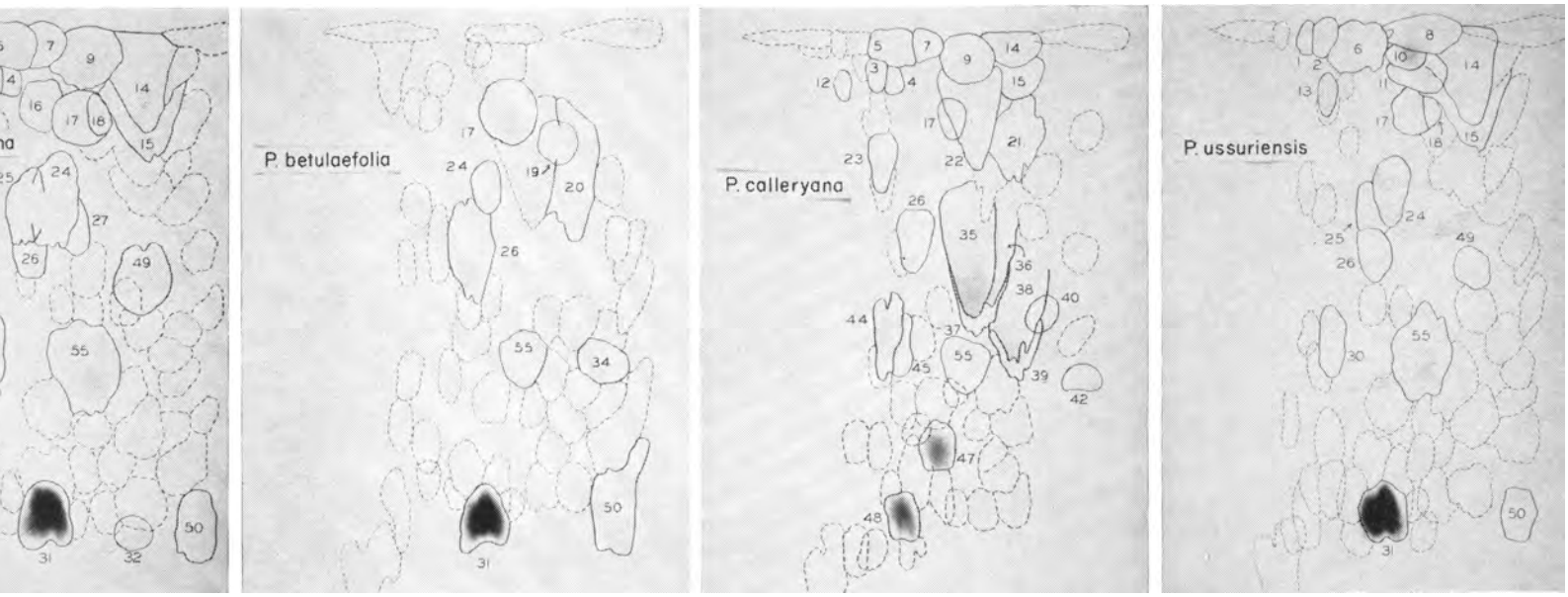
Sample collection

Samples were obtained from trees in variety and species collections at Davis, California, and at Medford and Corvallis, Oregon. Seedling populations were also sampled, including commercial orchards where some knowledge of rootstock identity was available. Samples were quick-frozen when obtained, to minimize chemical alteration, and extracted with methanol in the laboratory. Portions of the extracts were subjected to two-dimensional paper chromatography which separated the constituents of the extracts, as shown in the illustrations. The position of each compound (or spot), which depends upon its chemical nature and the solvents employed to effect its migration, was detected on the chromatograms by its appearance under ultraviolet light and by spraying with chromogenic reagents. Each spot was numbered and related to the species from which a given sample was obtained.

Bark extracts

Chromatographed extracts of bark from roots of different ages, and of trunk bark, possessed many substances, but three major components appeared related to species. Extracts of trunk bark between the graft union and the roots provided the best indication of species identity. With these samples, *P. communis* appeared distinguishable from the four oriental species. There were, however, a few exceptions where a sample of oriental species was not clearly separated from *P. communis*. While some division among the oriental species was possible, they could not all be individually identified. In addition, the oriental parents of two interspecific hybrids with *P. communis* were not detectable and the samples were erroneously identified only as *P. communis*.

Since bark extracts provided neither the specificity nor the assurance of accuracy desired, leaf extracts were evaluated. It was apparent after examination



ies Used as Rootstocks

of only a few samples that leaf extracts provided strikingly different chromatographic patterns for each of the five species being considered. While many components were common to all, there were also a number of spots which were either specific to a given species or which occurred in some but not all species. Thus, the five species could be characterized by both the presence or absence of specific constituents. Chromatograms representative of each of the species are illustrated. Spots were selected as being useful for identification after considering all available sources. These were: *P. communis*, 11 varieties and 39 seedlings; *P. serotina*, 12 varieties and 43 seedlings; *P. ussuriensis*, 20 varieties and 24 seedlings; *P. calleryana*, 11 clones and 7 seedlings; *P. betulaefolia*, 8 clones and 9 seedlings.

While the amounts of certain compounds were indicative of species, many were either present in high amounts or absent to the limit of detection. Several chemical types also existed within each species. These included groups of varieties or seedlings, but individual varieties were not identifiable to the exclusion of others within a species. Spots employed for identification were of constant appearance in extracts of mature leaves obtained at any time within a season and over several seasons.

Because of the high susceptibility of *P. serotina* to pear decline, it was of interest to determine whether characteristics of this species could be detected in hybrids with *P. communis*. Such identifications are impossible by morphological means. Chromatographed leaf extracts of Kieffer and Le Conte varieties—hybrids between *P. communis* and *P. serotina*—did in fact, possess components of each species. Controlled crosses were made using Bartlett, Winter Nelis, and two varieties of *P. serotina;* each as both pollen and seed parents. In every case—six plants of each cross—compounds from the *P. serotina* parent were present on chromatograms. Also, *P. communis* characteristics were readily found with most of these extracts, though with a few the *P. communis* parentage was difficult to establish. If any error were to be made with these hybrids it would be the uncertain identity of the *P. communis* parent.

Hybrid examination

Preliminary examinations of hybrids between *P. communis* and either *P. ussuriensis, P. calleryana* or *P. betulaefolia* indicate the same results as with *P. serotina*. Thus, if *P. communis* seedlings were F_1 hybrids with any of the five oriental species considered here, the oriental species parentage would be detectable.

There were instances of samples, from both species collections and orchards, where the chromatographic results did not agree with the reported identity. These exceptions clearly fit a different species or occasionally an interspecific hybrid. A few seedling populations, such as an orchard planting or a seed lot, were identified differently from that represented before chromatographic evaluation.

It is concluded that this chemical approach to identification of pear species and pear rootstocks is vastly superior to subjective estimates of identity based on leaf and shoot morphology. The components employed for identification here are under more direct genetic control than are morphological characteristics. These procedures provide means of detecting not only the presence of interspecific hybrids, but also the parents of that hybrid. The extent and reliability of identification based upon leaf extracts is so much greater than with bark extracts that only leaf samples are now being employed.

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