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Status of *Trifolium ambiguum* as a Forage Legume¹

By WAYNE F. KEIM

Trifolium ambiguum, more commonly known as Pellett clover, Honey clover, or Kura clover, is a legume possessing some highly desirable agronomic qualities (Figure 1). Most important of these

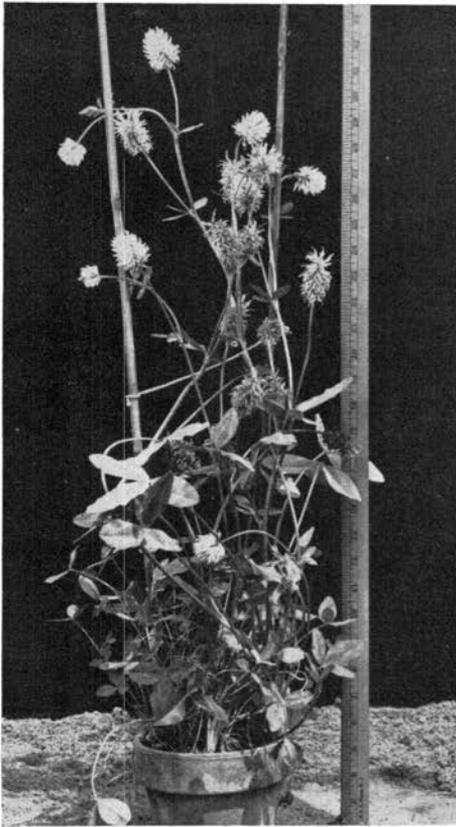


Figure 1. *Trifolium ambiguum*

qualities is its exceptional ability to spread extensively by rhizomes, an attribute significant in establishing a perennial forage legume. Secondly, it is highly attractive to the bees because of its high nectar content. Also, it is winter hardy and resistant to some serious clover diseases (9). Experiment station and soil conservation specialists have indicated a real interest in the species. Its extensive "root" system makes it valuable for soil conservation purposes. One S. C. S. nursery manager has an established field on a tract "too wet to grow alfalfa." Others indicate it is exceptionally drought resistant. As for its palatability it appears to be entirely satisfactory. Pellett (10) tells an interesting experience on this topic. Some cows from a field adjoining his honey plant gardens got into the gardens where many legumes were being tested for honey purposes. They found the plots of *T. ambiguum*, and pastured them cleanly.

¹Most of the author's research contribution to this paper was conducted at Cornell University.

One of the most complete and interesting descriptions of the species was supplied by the Russians (8) in 1945. They state that it is a valuable forage plant for use in perennial pastures, and that it has wide distribution in southern Russia. It is found in the river valleys up to the sub-alpine regions.

All descriptions so far indicate *T. ambiguum* to be the answer to the farmer's long needed perennial legume. However, the most serious of all legume disadvantages occurs in this species. No *Rhizobium* bacterium species is known which will react symbiotically with *T. ambiguum*, and thus the plant is unable to fix nitrogen. The small nodules that are sometimes found do not contain nitrogen-fixing bacteria. A legume with this stigma is of little value to the farmer.

The first line of attack to solve the situation would be to check all existing strains of rhizobia for effective activity on *T. ambiguum*. This possibility has been thoroughly investigated by the University of Wisconsin soil bacteriologists (1, 9) and by the U.S.D.A. soil bacteriologists at Beltsville. At Wisconsin about 50 strains of rhizobia were isolated from *T. ambiguum* and tested on the host plant under controlled conditions. In addition about 300 rhizobia from various other legumes were tested. Although some very interesting and significant results were obtained from these studies, yet no strains of rhizobia were located which were effective on *T. ambiguum*.

Considerable thought has been given to this dilemma by this investigator and others. Marschall von Bieberstein (2) first described *T. ambiguum* in 1808, but investigators in the United States were almost completely unaware of the species until 1941 when Pellett received a few seeds from the Caucasus area and planted them in his test gardens. The results of this planting were so overwhelmingly successful that he called it "a legume unlike any previously known to agriculture" (11).

All available seeds from Pellett's planting were given to experiment station workers for additional research. Beekeepers had to be satisfied with vegetative "root" divisions. The author's effort concerned those beekeepers in New York state who obtained vegetative divisions from Pellett. It was believed that there might be a remote possibility that one or more of these persons might have an established plot upon which the local rhizobia might be effective. Letters were sent to 14 of these beekeepers inquiring into the success they had experienced. Six answered the inquiry, and

the results were the same; plantings at first looked promising, but later on "yellowed up" and died.

The second line of attack was directed toward the native habitat of the species, Russia. If the species had survived in Russia for a century and a half after it was first described, it seemed reasonable that an effective strain of rhizobia might be present in the legume's native habitat. In the lengthy description of the species given by the Russians in 1945, as previously cited, no mention was made that the nitrogen-fixation problem did exist there. In October 1951 letters were written to the Botanical Institutes in Moscow and Leningrad, inquiring into the problem. No answers were received; there is no way to know even if the letters reached their destination.

A third line of attack centered on northeastern Turkey near the Russian border. The same Russian publication cited villages in northeastern Turkey also as being areas of native habitat for *T. ambiguum*. A former colleague was contacted in Turkey and the problem presented to him. Plans were formulated and transportation was arranged by him with the Turkish Ministry of Agriculture for an expedition to northeastern Turkey. It was necessary to wait until April or May of the next year because of the snow in that area. However, unusual difficulties were encountered, which is a story in itself, and the expedition was not carried out in the spring of 1952 as planned.

However, in September of 1952 an agronomist from the University of Ankara in Turkey, and a plant exploration man from the United States travelled in the region of eastern Turkey and collected seeds, soils, and root parts of various legumes. This material was sent to Beltsville, where it remained for test by U.S.D.A. soil bacteriologists. These tests are still in progress; however, results will be forthcoming soon.

The above methods of approach were the most direct and the most likely to attain significant results. However, several alternate attacks have been made. One was the unique approach reported last year by Hely and his colleagues in Belgium (6). They grafted *Trifolium repens* on the seedlings of *T. ambiguum*. Four general types of grafts were used, and all methods gave a high percentage of success. When either plant tops or roots of *T. repens* were grafted on to *T. ambiguum*, relatively large and healthy nodules were produced on *T. ambiguum*. Ungrafted plants of *T. ambiguum* grown in the same pots with the above produced no nodules. Isolations from these nodules have been cultured, but it

is as yet unknown if direct inoculation of these rhizobia into *T. ambiguum* will be effective. This research is in progress at present and additional results will be reported later.

In Australia, where Hely is now located, several people are interested in the fundamental nature of the problem (5); why should a particular species of a genus which normally nodulates so well, be apparently unable to nodulate effectively? The problem is being seriously attacked.

Another alternate approach to the nodulation obstacle was that of interspecific hybridization, an attack taken by the author (7). It had been shown clearly by the outstanding histological studies of Guravich (4) at Wisconsin that *Trifolium ambiguum* and *T. hybridum*, alsike clover, were compatible to the extent that fertilization occurred, and that normal-appearing hybrid embryos began development. However, abortion of the hybrid embryos occurred at various stages of development, and only shrivelled, inviable seeds were produced.

Embryo culture techniques were applied to this interspecific cross of *T. ambiguum* x *T. hybridum*, and 22 F₁ hybrids were obtained. The validity of the cross was cytogenetically ascertained, since the F₁ had a 2n chromosome count of 32 as compared to the 2n = 48 count of *T. ambiguum* and the 2n = 16 count of *T. hybridum*. Many of these plants appeared to have a physiological unbalance, and one by one they died until only five are still alive. Five of the F₁ plants have flowered and all exhibited both male and female sterility.

It was hoped that some of the F₁ plants perhaps would possess the rhizomatous character of *T. ambiguum* and the ability to fix nitrogen of *T. hybridum*. Effective backcrossing could then be applied and real progress attained. Besides the sterility obstacle of the F₁ plants, none of them exhibited effective nodulation when the soil was inoculated with a commercial inoculant effective on *T. hybridum*.

Research is in progress now in an attempt to overcome the sterility obstacle. The chromosome complements of the parental species have been doubled and crosses will be performed at the 4n level. This approach was successful on another *Trifolium* interspecific cross (3).

An interesting sidelight to the main problem cropped up during this study, the refusal of *T. ambiguum* to flower in the fall and winter under greenhouse conditions. Numerous nonorthogonal treatments were applied to the species, involving day length, tem-

perature, moisture, nitrogen level, and age of plants. Day lengths of 10, 14, 18 and 22 hours were initiated. Under each of these day lengths were placed old plants and young seedlings, some of which had been in the greenhouse continually and others in the cold frame over winter. Various nitrogen levels were applied. On some plants excessive water was applied. Later, additional temperature treatments were applied using temperatures of 55° to 60° F. and at 40° F. From the entire group of treatments herein briefly described, involving 300 plants, only one plant produced flowers between September 15 and March 1. Beginning about March 15, plants from nearly all of the many treatments began to flower, and by May 15 many plants were in flower. No flowering difficulties were encountered when the species was grown in the field. Correspondence with Guravich at Wisconsin, Trimble at Penn State, and Hely in Belgium indicates each has had a similar experience with the species. This problem should be of interest to the physiologist, and it no doubt will be attacked by one soon. A solution to the flowering problem would be a real contribution. Legume breeders will refuse to work with the species in the greenhouse if they are continually frustrated by its refusal to flower there.

It has been a pleasure to present to you my viewpoints on *Trifolium ambiguum*. It is my belief that we will hear much of this legume in the coming years.

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