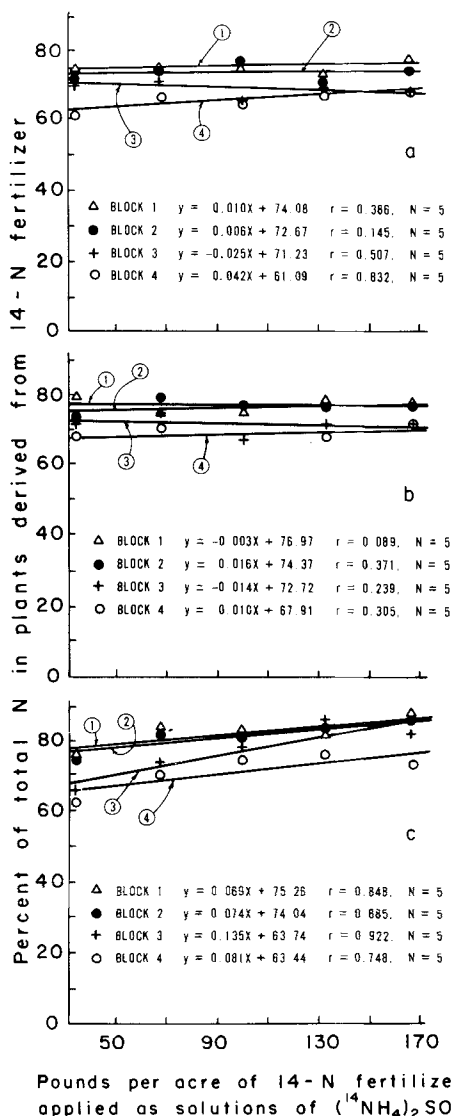


These Kearney Field Station experiments represent one of the opening phases in a new era for tracing the routes taken by nitrogen after it is applied in the field as a fertilizer. They have been made possible by the recent breakthrough in very low-temperature cryogenic techniques whereby nitric-oxide gas (NO) is liquified and then distilled to separate the stable 14-N and 15-N isotopes that occur in nature. Thus a long-standing technological gap has been closed so that isotopic-N tracing techniques of the kind reported here may become common in the future.

These experiments are planned for five consecutive years of fertilization with traceable 14-N. Thereafter, tracing can be continued into the indefinite future without further additions of tracer nitro-

Contributions of fertilizer-N to plant-N: (a) 11 days after emergence of maize seedlings; (b) 16 days after emergence of maize seedlings; and (c) 27 days after emergence of maize seedlings.



gen. Thus future questions relating to long term nitrogen uptake by crop plants may be answerable from these Kearney Field Station plots.

TABLE 2. PRECISION AND SENSITIVITY OF ISOTOPIC 14-N IN EARLY TRACING OF FERTILIZER-N UPTAKE BY MAIZE PLANTS

(A) Overall mean percentages of contained fertilizer-N found in plant-N at 11, 16 and 27 days						
Days after emergence	11	16	27			
% contained fertilizer in plant-N	70.6	73.2	78.3			
(LSD .05 = 5.38; LSD .01 = 7.17)						
(B) Overall mean percentages of contained fertilizer-N found in plant-N from various fertilizer-N treatments (all replications and all sample dates)						
Pounds of isotopic 14-N applied per acre	0	33	67	100	133	167
% contained fertilizer-N in plant	0	70.6	74.8	73.8	75.1	76.0
(LSD .05 = 4.17; LSD .01 = 5.56)						
(C) Overall mean percentages of contained fertilizer-N found in plant-N for all sample dates and all fertilization rates except zero.						
Block number	1	2	3	4		
% contained fertilizer-N in plants	77.8	76.9	72.4	68.9		
(LSD .05 = 4.66; LSD .01 = 6.22)						

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Weather effects on baits for controlling EUROPEAN BROWN GARDEN SNAILS IN CITRUS

J. L. PAPPAS · G. E. CARMAN · G. F. WOOD

Weather conditions following bait treatments for the control of the European brown garden snail in citrus groves substantially influenced the ultimate effectiveness of carbamate molluscicides. Baits containing Mesuro were more effective under unfavorable conditions than the other carbamates used in the tests. Under optimum treatment conditions, all of the carbamate baits were effective, particularly those with metaldehyde inclusions.

SINCE THE CARBAMATE INSECTICIDE Isolan was first reported to possess molluscicidal properties, and with subsequent discoveries that several other carbamate compounds are also effective against mollusks, particularly snails, it has become increasingly apparent that the total effectiveness of these compounds is appreciably influenced by weather conditions during and immediately following their application. These observations have led to the usage of such warnings as "carbamate-based molluscicides should

be applied during periods of high snail activity when warm drying weather following treatment is anticipated," or that "many treatment-affected snails, afforded protection of shade and dampness following treatment, may recover." While these statements were supported by repeated field observations, the actual differential of control achieved by similar carbamate treatments, when applied just before drying or non-drying weather conditions, had not been viably demonstrated in cooperative field trials. Although such comparisons could not be purposefully arranged, field tests undertaken in the spring of 1971, and in the spring of 1972 (involving comparable treatments) happened to provide such a comparison.

Immediately following the 1971 test applications, an unpredicted storm system moved into the general area. While no measurable precipitation fell, there was a heavy overcast and morning drizzle for a week following treatment applications. When the weather did clear, there were several more days that remained cold because of wind conditions. Conversely, following the 1972 test applica-

COMPARISON OF FIELD TEST RESULTS FOR CONTROL OF EUROPEAN BROWN SNAIL INFESTING CITRUS, AS INFLUENCED BY WEATHER CONDITIONS FOLLOWING TREATMENT APPLICATIONS

Materials	(Per cent composition)	Non-drying* Drying†	
		4-16-71	5-18-72
		Mortality	
	%	%	%
Carzol-apple pomace	(2-98)	49.1	74.9
Furadan-metaldehyde-apple pomace	(2-2-96)	79.4	93.2
Furadan-apple pomace	(2-98)	52.5	81.1
Furadan-metaldehyde-apple pomace	(2-2-96)	63.3	88.7
MesuroI-apple pomace	(2-98)	82.1	96.8
MesuroI-metaldehyde-apple pomace	(2-2-96)	76.6	94.5
MesuroI-bran	(2-98)	87.6	93.5
MesuroI-metaldehyde-bran	(2-2-96)	79.0	—
Lannate-apple pomace	(2-98)	40.2	81.2
Lannate-metaldehyde-apple pomace	(2-2-96)	67.3	90.2
Lannate-bran	(2-98)	52.8	91.8
Lannate-metaldehyde-bran	(2-2-96)	68.1	—
Zectran-apple pomace	(2-98)	—	79.9
Zectran-metaldehyde-apple pomace	2-2-96)	—	97.9
Tricalcium arsenate-metaldehyde-bran	(5-3-92)	94.9	92.3

* Treatment followed by several days of cloudy weather. When clearing did occur, the wind blew and days were abnormally cold.

† Treatment followed by light shower after which days were sunny and warm.

tions, approximately 0.1-inch of rain fell on the evening of the day the treatments were made, after which sunny warm days prevailed for the duration of the test.

This report presents the comparative data from these two tests for the control of the European brown garden snail, *Hilix aspersa* L., with carbamate molluscicides which additionally show the apparent influence of weather conditions following treatment on the degree of snail control achieved.

The materials used in both tests were Carzol, Furadan, MesuroI and Lannate. They were compared with a standard proprietary bait containing 5.0% tricalcium arsenate and 3% metaldehyde on a bran substrate. Although not available for the 1971 test, Zectran was included in the 1972 trials and the data are provided for informational purposes. All materials were formulated as 2% baits on apple pomace substrate both with and without the inclusion of 2% metaldehyde. In addition, MesuroI and Lannate were formulated at the 2% rate on a bran substrate, both alone, and in combination with 2% metaldehyde.

The 1971 treatments were applied to grapefruit trees in Corona, California. Sixteen trees in a 4 × 4 block comprised each treatment area. Mortality counts were obtained from the center four trees of each treatment block. The baits were applied at the rate of 0.5-lb per tree.

The 1972 treatments were applied to tangerine trees in Fallbrook, California. Each treatment plot consisted of 24 trees

in a 4 × 6 block and baits were applied at the rate of 0.33-lb per tree. The per tree dosage was reduced because the trees were smaller and were spaced at 10-ft intervals in the rows. Four count-trees were selected from the center of each treatment block.

In each instance, post treatment counts were obtained 2 weeks following bait applications. Snails under each count-tree were examined and the number of live and dead recorded. Results of the tests are presented in the table. Examination of these data reveals several points for consideration.

In a direct comparison of the 1971 and 1972 test results, it was apparent that the overall performance of the baits was more favorable in 1972. Except for the standard proprietary arsenical bait, the increases in mortality in the 1972 test ranged from a low of 5.9% to a high of 41% with a mean average increase of approximately 20%.

It should also be noted that the highest mortalities were obtained by the use of baits in which metaldehyde had been included. Mortality was substantially increased in every instance by the inclusion of metaldehyde except in baits contain-

ing MesuroI. The lower mortalities obtained with the MesuroI-metaldehyde baits in these tests may be of some significance but further trials should be undertaken.

In these tests MesuroI demonstrated definite superiority over Carzol, Furadan, Lannate, and Zectran as a molluscicide, particularly with regard to its ability to function creditably even when post application weather conditions were adverse. The potential of this compound was first recognized in 1963 in a series of preliminary field tests. The continuing development of supportive experimental and field evidence on the performance of MesuroI was then delayed because of a temporary withdrawal of the compound from further consideration by the sponsoring chemical company.

Similarly, Zectran had been withdrawn from further consideration following preliminary tests during the 1960's and was not reactivated for commercial development until the spring of 1972. In the earlier tests as well as in the 1972 spring test, Zectran appeared to be a useful molluscicide but notably when used in combination with metaldehyde, being more favored by the inclusion of the

CITRUS STUBBORN DISEASE CULTURED FROM BEET

ING MING LEE G. CARTIA E. C. CALAVAN

The citrus stubborn disease organism, *Spiroplasma citri*, has been cultured from beet leafhoppers collected from citrus near Riverside. This leafhopper is commonly a vector of curly top virus of sugar beets and other plants and is periodically abundant in hot, dry areas where sugar beets, citrus, and many other hosts are grown. This is the first report of a natural insect carrier of the citrus stubborn organism and is believed to be the first recorded instance of culturing a naturally acquired mycoplasma-like organism from an insect carrier.

CITRUS STUBBORN DISEASE has spread for several decades in arid areas of California and many other arid citrus producing areas of the world. In California an estimated two million orange, grapefruit, and tangelo trees are severely

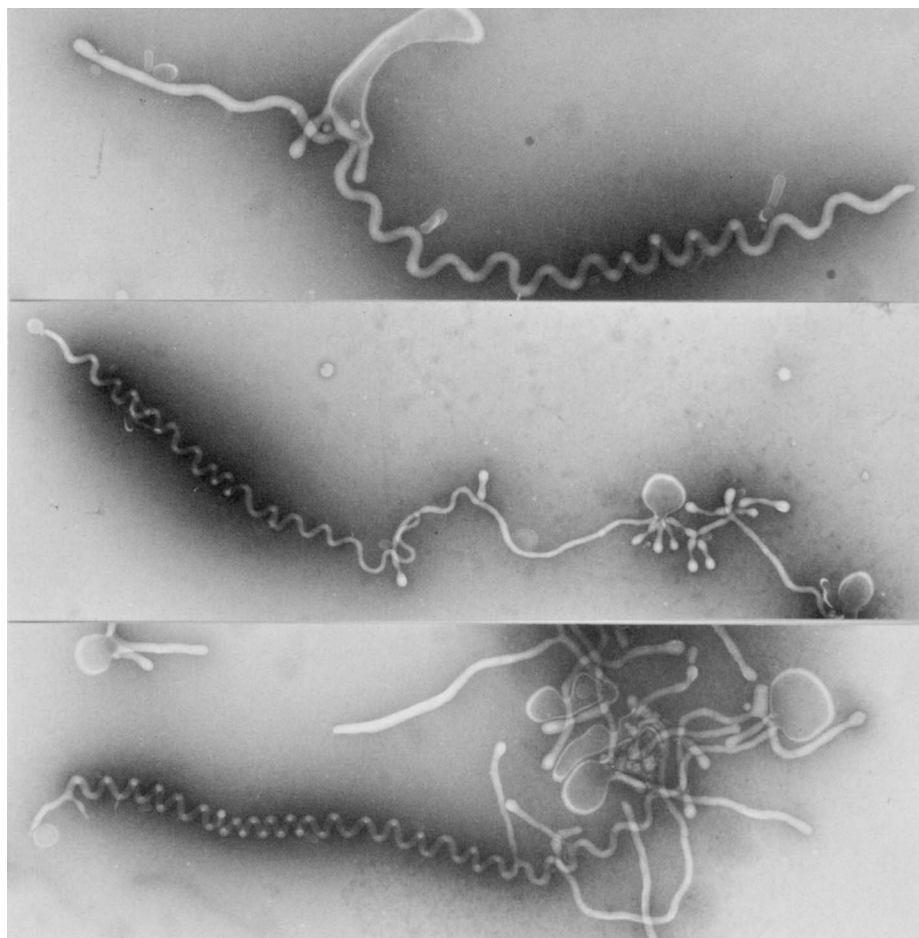
affected to the point of being worthless. Many other trees may be infected in one or several branches without being severely damaged.

Research aimed at finding the vector of stubborn disease was begun by entomologists about twenty years ago and has continued without success until now. Four years ago it was learned that the cause of stubborn is not a virus, as presumed earlier, but is apparently a mycoplasma-like organism, now called *Spiroplasma citri*, found in the sieve tubes of stubborn-diseased citrus phloem. In 1970 scientists discovered here, and independently in France, that *S. citri* could easily be cultured from phloem of stubborn-diseased citrus and maintained indefinitely in cell-free culture media. The capability of growing the stubborn organism in culture in the laboratory led to the development of a new method of searching for the vector—that is, of cul-

latter than were Carzol, Furadan or Lan-nate.

In both instances, the standard propri-etary treatment containing 5% trical-cium arsenate and 3% metaldehyde in a pelletized bran substrate gave good control. This ability to function in a wider range of weather conditions during the postapplication period suggests that by ingestion of the bait, the snail receives a sufficient quantity of toxicant to produce death by poisoning. While snails may also be killed by ingesting carbamate-based baits, a great number of the feeding snails may become temporarily paralyzed by contact with the toxin before they have ingested a lethal amount. These are the snails that are affected by weather conditions following treatment. Those that are subjected to warm, drying conditions, usually die from dessication while many of those that are afforded dampness and protection from weather elements are able to recover from their sublethal ex-posure to the toxicant.

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Top photo: Single young cell of *Spiroplasma citri* from culture derived from beet leafhopper. Note sacklike "bleb" and spiral filament. X22,000 diameters. Middle: Cells of *S. citri* from a 5-day-old culture from a bee leafhopper. X14,000 diameters. Bottom: Cells of *S. citri* from 5-day old culture derived from stubborn diseased sweet orange shoot. X15,000 diameters.

THE ORGANISM OF THE BEET LEAFHOPPER

G. H. KALOOSTIAN

turing from insects found on or near stubborn-diseased citrus trees.

Leafhoppers and psyllids are known to be the vectors of a number of mycoplasma-like diseases of plants, which prompted us to concentrate our efforts on leafhoppers present in an experimental block of young Madam Vinous sweet orange seedlings at the University of California Moreno Farm, east of Riverside. Extensive natural spread of stubborn disease into healthy plants was noted in this block in 1971, 1972 and 1973.

Spiroplasma citri was repeatedly cultured from beet leafhoppers, *Circulifer tenellus* (Baker). The leafhoppers were collected from sweet orange seedlings and weeds at the Moreno Farm in August and September 1973 (see photo). Leafhoppers were collected with an aspirator, killed in the laboratory, washed in 1% sodium hypochlorite, rinsed in sterile distilled water, finely ground in a special

broth medium and placed in 0.45 micron filter units. The filtrate was collected in sterile flasks, after which aliquots were added to special agar plates and incubated at 86°F. Material from the flasks and plates was examined by light or electron microscopy 5-10 days later to determine presence or absence of the *Spiroplasma*.

The discovery of *S. citri* in beet leafhoppers is highly significant, because it identifies a probable natural vector for stubborn disease. Moreover, *C. tenellus* is a migratory insect that periodically builds up high populations in hot desert areas of California and Arizona, where stubborn disease is common. The beet leafhopper is also the recognized vector of curly top virus of sugar beet and other plants, but sometimes feeds on citrus. A very close relative, *Circulifer opacipennis* (Lethierry), is common in the Mediterranean area and is an important vector of the Turkish strain of curly top virus of sugar beets in Turkey, where citrus stubborn disease is also severe.

In California, stubborn is principally a disease of young citrus trees and can

be best prevented by propagating from healthy material indoors or in a cool area where few if any beet leafhoppers occur. If *C. tenellus* should prove to be the only vector of *S. citri* in California, its control would prevent the natural spread of stubborn disease.

The beet leafhopper *C. tenellus* from the Moreno Farm was identified by M. W. Nielson and J. P. Kramer, USDA, ARS, Forage Insects Research Laboratory, Tucson, Arizona, and Systematic Entomology Laboratory, Washington, D.C., respectively.

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