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荧光物示踪法定量测定水稻-叶蝉-蜘蛛食物链的营养关系

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摘要：首次采用稀土元素铕荧光物示踪法，对水稻-叶蝉-蜘蛛这一食物链营养关系进行了初步的定量测试。结果表明，所选用的稀土元素铕可以通过该食物链而传递，证明铕用于食物链营养关系定量分析是可行的；早稻孕穗期、晚稻分蘖期施铕 7d 后，其含铕量均表现为叶片＞茎及叶鞘；根据叶蝉取食水稻后测定的含铕量和叶蝉体重的不同进行折算，每 100 头叶蝉每天可取食水稻 0.71～1.13g；根据拟水狼蛛、锥腹肖蛸、食虫沟瘤蛛捕食叶蝉后含铕量计算，每头拟水狼蛛、锥腹肖蛸、食虫沟瘤蛛平均每天捕食叶蝉分别为 4.63 头、2.81 头、1.0 头。捕食能力：拟水狼蛛＞锥腹肖蛸＞食虫沟瘤蛛。

关键词：稀土元素；荧光物示踪；水稻-叶蝉-蜘蛛；食物链；营养关系

Quantitative Measurement of Nutritive Relationship of the Food Chain: Rice plant-leafhoppers-spiders by the Fluorescence Labeling Method

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Abstract: The rice leafhopper is one of the main paddy pest species in China, while the paddy spider is the primary predator of the rice leafhopper. In the past, the degree of damage that suffered as a result of paddy pests and the control manifested by paddy spiders toward pests was generally determined by the qualitative research method, which can hardly accurately reflect the dangerous degree of the pest toward the rice and the controlling ability of the spider toward the pest. This research focuses primarily on the quantitative measure of the nutritive relationship in the food chain among the rice plant, leafhoppers and spiders of the dominant species. By adopting lanthanide-europium as a fluorescent tracer for the first time, this test aims to provide the theoretical basis for further discovery of the function of the spider community's control over leafhoppers' reproduction.

Research materials　The rice plant, leafhoppers (*Nephotettix bipunctatus*), dominant spider species: *Pirate subpiraticus*, *Tetragnatha japonica*, *Ummelita insecticeps*, Eu_2O_3, etc.

In this experiment by planting early rice and late rice during the 2000 season, the following results were obtained:

The chosen lanthanide-europium was used in the experiment in which the dissolved europium in the soil was absorbed by the rice plant. Leafhoppers live on the plant while spiders prey on the leafhoppers for food. So the feasibility of using the fluorescent tracer in the quantitative analysis of the nutritive relationship in the food chain had been proven.

Seven days after the europium was applied to the soil, the content of the europium in the rice plant

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was measured. In the early rice plant during its pregnant stage , each gram of fresh stem and leaf contained europium at 1.799μg and 2.610μg, respectively. Whereas, during the middle of tiller period of the late rice plant, each gram of fresh sheath and leaf contained europium at 1.701μg and 3.10μg, respectively. The content of europium in the leaf was always more than that in the stem or in the sheath.

Measuring the amount of europium in the leafhoppers who lived on early rice plant and late rice plant after three days, each gram of fresh insects contained europium at 17.456μg and 13.64μg levels respectively. Based on the total weight of 100 leafhoppers (428mg in early rice, 373mg in late rice), then each group of 100 leafhoppers contained europium at 7.475μg in early rice and 5.074μg in late rice 100 leafhoppers ate 1.13g early rice plant and 0.71g late rice plant every day by estimation.

Tested spiders were fed leafhoppers. After three days, the average amount of europium content in each gram of fresh spiders *Pirate subpiraticus*, *Tetragnatha japonica* and *Ummelita insecticeps* was 7.55μg, 4.12μg and 11.96μg, respectively. Each gram of spiders contain 50 *Pirate subpiraticus* or 45 *Tetragnatha japonica* or 370 *Ummelita insecticeps*. 15.10μg, 9.17μg and 3.23μg of europium were found in 100 spiders of *Pirate subpiraticus*, *Tetragnatha japonica* and *Ummelita insecticeps* respectively. Therefore, the individual number of eaten leafhoppers by *Pirate subpiraticus* or *Tetragnatha japonica* or *Ummelita insecticeps* in one day was 4.63 or 2.81 or 1.0, respectively.

The predatory ability of the spider *Pirate subpiraticus* against leafhoppers is superior to that of *Tetragnatha japoica*, while *Tetragnatha japoica's* ability is superior to that of *Ummelita insecticep*.

Key words: lanthanide; fluorescent tracking; rice plant-leafhopper-spider; food chain; nutrition relationship

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稻叶蝉是我国水稻上的主要害虫之一,稻田蜘蛛是稻叶蝉的主要捕食性天敌。过去判断稻虫对水稻的危害程度和测定蜘蛛对稻虫的控制能力,基本上都是籍定性的方法进行研究,难以真实反映稻虫的危害程度和蜘蛛对稻虫的捕食功能[1]。在国家自然科学基金重点项目的资助下,作者于 2000 年首次采用稀土元素铕荧光物示踪手段,对水稻-黑尾叶蝉-优势种蜘蛛这一食物链的营养关系进行了初步的定量分析,旨在为进一步揭示蜘蛛群落控制黑尾叶蝉发生发展的功能提供理论依据[2~5]。

1　化学药品与仪器设备

1.1　化学药品

硝酸、盐酸、高氯酸、过氧化氢、抗坏血酸、硫氢化铵、磺基水杨酸、乙酸、氧化铕(Eu_2O_3)、等 20 余种。

1.2　仪器设备

1.2.1　主要仪器设备　万分之一电子分析天平、日本岛津 RF-5301PC 型荧光分光光度计及配套设备、恒温干燥箱、中华电阻炉(马弗氏炉)、pHS-3C 酸度计。

1.2.2　其他设备　坩埚、分液漏斗、烧杯、移液管、滴管、玻棒、电炉、排风扇、容量瓶、洗瓶、定量滤纸、pH 试纸、栽培盆及纱罩等。

2　材料与方法

2.1　研究材料

盆栽水稻(每盆 3 蔸);叶蝉(黑尾叶蝉 *Nephotettix bipunctatus*);优势种蜘蛛:狼蛛(拟水狼蛸 *Pirata subiraticus*)、肖蛸(锥腹肖蛸 *Tetragnatha japonica*)、微蛛(食虫沟瘤蛛 *Ummeliata insecticeps*)、氧化铕(Eu_2O_3)溶液等。

2.2　研究方法

2.2.1　试验处理　设铕标记和空白对照　铕标记　设水稻-叶蝉-拟水狼蛛、水稻-叶蝉-锥腹肖蛸和水稻-叶蝉-食虫沟瘤蛛 3 个组合,各重复 3 次,用 15μg /ml 铕溶液标记水稻(7d 后,取水稻样品)+ 叶蝉(3d 后)

＋蜘蛛(3d 后,结束实验)。在投放叶蝉时,每盆采用直径为 25cm,高 80cm,下部活口扎紧,侧面装有拉链的尼龙网罩罩住。

空白对照　设上述 3 个组合,各重复 2 次,未用铕标记水稻(7d 后,取水稻样品)＋叶蝉(3d 后)＋蜘蛛(3d 后,结束实验)。在投放叶蝉时同样用尼龙网罩罩住。

2.2.2　样品采集处理　分别采取铕标记和空白对照水稻(地上部分)10～20g,每一批实验结束时,采集各组合叶蝉、蜘蛛,将样品分别计数,称湿重得鲜重,烘干后得干重,编号保存备用。

2.2.3　测定方法　采用本研究室最近探索的稀土元素铕荧光物示踪定量检测法(见中华人民共和国国家知识产权局专利公开号 CN 1316642A):①样品灰化或硝化;②铕分离萃取;③荧光分光光度计测定,将待测液置于岛津 RF-5301PC 型荧光分光光度计中以激发波 390nm 测定样品中铕的发光强度,详细记录;④数据整理,按标准曲线计算样品中铕的含量(标准曲线为 $y=5.25+297.58x$),定量分析各营养层的关系。

2.2.4　样品中含铕量计算

每克鲜重样品含铕量＝荧光强度/297.58×30÷鲜重(30 为测定时样品液的稀释倍数)

平均每克鲜重样品含铕量＝重复样品含铕量之和/重复次数

实际每克鲜重样品含铕量＝平均每克鲜重样品含铕量－空白对照样品含铕量

3　结果与分析

经过 2000 年早、晚稻两季盆栽网罩荧光物铕示踪试验,初步获得了水稻-叶蝉-蜘蛛之间营养关系。

3.1　水稻吸收荧光物铕的情况

结果表明,所选用的稀土元素铕可以通过施用于泥土被水稻吸收,而且铕在水稻的不同部位分布含量不同。早稻孕穗期施铕 7d 后,其每克鲜重茎、叶中的含铕量分别为 1.799μg 和 2.610μg;晚稻分蘖中期施铕 7d 后,其每克鲜重茎(此时为叶鞘)、叶中的含铕量为 1.701μg 和 3.10μg(见表 1)。早稻和晚稻,其铕的含量均表现为叶片＞茎及叶鞘,而且早、晚稻茎、叶中的含铕量比较接近。

表 1　水稻标记 7d 后的含铕量测定结果

Table 1　The amount of rare earth element Eu in the rice-plant after marking it 7 days

处理 Treatment	材料 Materials	生育期 Stage of development	鲜重(mg) Wet mass	荧光强度 Intensity of fluorescence	每克鲜重含铕量(μg) μg of Eu/g, wet mass	实际每克鲜重含铕量(μg) Net μg of Eu/g, wet mass
铕标记 Marked by Eu	早稻茎 Early rice stem	抽穗 Heading stage	1320	29.159	2.227	1.799
空白对照 Control	早稻茎 Early rice stem	抽穗 Heading stage	1320	5.68	0.428	
铕标记 Marked by Eu	早稻叶片 Early rice leaf blade	抽穗 Heading stage	930	28.286	3.068	2.610
空白对照 Control	早稻叶片 Early rice leaf blade	抽穗 Heading stage	924	4.194	0.458	
铕标记 Marked by Eu	晚稻叶鞘 Late rice leaf sheath	拔节 Jointing stage	900	19.950	2.222	1.701
空白对照 Control	晚稻叶鞘 Late rice leaf sheath	拔节 Jointing stage	900	4.65	0.521	
铕标记 Marked by Eu	晚稻叶片 Late rice leaf blade	拔节 Jointing stage	568	20.17	3.710	3.100
空白对照 Control	晚稻叶片 Late rice leaf blade	拔节 Jointing stage	568	3.434	0.610	

3.2　叶蝉危害水稻的定量分析

放虫 3d **后对叶蝉进行测定（结果见表** 2），**早稻期每克鲜重叶蝉的含铕量为** 14.51～20.68μg，**平均为** 17.465μg；**晚稻期每克鲜重叶蝉的含铕量为** 6.805～21.667μg，**平均为** 13.64μg。**早稻期按每** 100 **头叶蝉平均重** 428mg **计算，其铕的含量为** 7.475μg（**即** 17.465μg/g×428mg），**估计每** 100 **头叶蝉每天可取食水稻** 1.13g{**即** 7.475μg÷[（1.799 **茎**＋2.61 **叶**）÷2]μg/g÷3d}。**晚稻期按每** 100 **头叶蝉重** 372mg **计算，其铕的含量为** 5.074μg（**即** 13.64μg/g×372mg），**估计每** 100 **头叶蝉每天可取食水稻** 0.71g{**即** 5.074μg÷[（1.701＋3.10）÷2]μg/g÷3d}。**结果表明，早稻期叶蝉的取食量较晚稻期叶蝉的为大，究其原因，可能主要是由于早稻期叶蝉较晚稻期叶蝉个体大（早稻期每** 100 **头叶蝉重量多** 56mg）**所致，个体大则取食量亦大。**

表 2　叶蝉取食 3d 后的含铕量测定结果

Table 2　The amount of rare earth element Eu in the marked leafhoppers' body after feeding 3 days

处　理 Treatment	材　料 Materials	生育期 Stage of development	数量（头） Num. (ind.)	鲜重（mg） Wet mass	荧光强度 Intensity of fluorescence	每克鲜重含铕量（μg） μg of Eu/g, wet mass	实际每克鲜重含铕量（μg） Net μg of Eu/g, wet mass
铕标记 Marked by Eu	早稻叶蝉 The leafhopper on early rice	3 龄至成虫 From 3th instar larva to adult	13	45.8	15.663	34.229	17.196
空白对照 Control	早稻叶蝉 The leafhopper on early rice	3 龄至成虫 From 3th instar larva to adult	7	26.8	4.679	17.033	
铕标记 Marked by Eu	早稻叶蝉 The leafhopper on early rice	3 龄至成虫 From 3th instar larva to adult	11	48.4	15.191	31.673	20.689
空白对照 Control	早稻叶蝉 The leafhopper on early rice	3 龄至成虫 From 3th instar larva to adult	8	39.7	3.850	10.984	
铕标记 Marked by Eu	早稻叶蝉 The leafhopper on early rice	3 龄至成虫 From 3th instar larva to adult	9	39.8	10.549	26.76	14.51
空白对照 Control	早稻叶蝉 The leafhopper on early rice	3 龄至成虫 From 3th instar larva to adult	8	38.4	4.677	12.25	
铕标记 Marked by Eu	晚稻叶蝉 The leafhopper on late rice	5 龄、成虫 5th instar larva and adult	17	60.6	15.028	26.927	21.667
空白对照 Control	晚稻叶蝉 The leafhopper on late rice	5 龄、成虫 5th instar larva and adult	20	74.6	3.893	5.260	
铕标记 Marked by Eu	晚稻叶蝉 The leafhopper on late rice	5 龄、成虫 5th instar larva and adult	20	72.9	7.420	10.25	6.805
空白对照 Control	晚稻叶蝉 The leafhopper on late rice	5 龄、成虫 5th instar larva and adult	20	68.8	2.351	3.445	
铕标记 Marked by Eu	晚稻叶蝉 The leafhopper on late rice	5 龄、成虫 5th instar larva and adult	20	84.8	13.517	15.085	12.448
空白对照 Control	晚稻叶蝉 The leafhopper on late rice	5 龄、成虫 5th instar larva and adult	20	72.1	1.886	2.637	

3.3 蜘蛛对叶蝉的捕食效应

由表 3 可知，投入蜘蛛 3d 后测定，早稻期折合每克鲜重拟水狼蛛、锥腹肖蛸、食虫沟瘤蛛含铕量分别为 10.086μg、4.696μg、11.88μg，晚稻期分别为 5.015μg、3.559μg、12.028μg，每克鲜重蜘蛛的含铕量均表现出食虫沟瘤蛛＞拟水狼蛛＞锥腹肖蛸的趋势。就体重而言，每克鲜重拟水狼蛛约为 50 头（平均 20mg/头）、锥腹肖蛸约为 45 头（平均 22.2mg/头），食虫沟瘤蛛则有 370 头（平均 2.7mg/头）；若以每 100 头蜘蛛的含铕量计算，早稻期每 100 头拟水狼蛛、锥腹肖蛸、食虫沟瘤蛛的含铕量分别为 20.172μg、10.436μg、3.21μg，晚稻期分别为 10.03μg、7.908μg、3.25μg。由此可以计算出早、晚稻试验期间每头拟水狼蛛、锥腹肖

表 3 蜘蛛投放 3d 后的含铕量测定结果

Table 3 The amount of rare earth element Eu in the marked spiders' body after feeding 3 days

处 理 Treatment	材 料 Materials	生育期 Stage of development	数量(头) Num. (ind.)	鲜重(mg) Wet mass	荧光强度 Intensily of fluorescence	每克鲜重含铕量(μg) μg of Eu/g, wet mass	实际每克鲜重含铕量(μg) Net μg of Eu/g, wet mass
铕标记 Marked by Eu	早稻狼蛛 The spiders pirata in early rice field	成、亚成蛛 From adult to subadult	4	64.7	9.424	16.469	10.086
空白对照 Control	早稻狼蛛 The spiders pirata in early rice field	成、亚成蛛 From adult to subadult	3.5	61.6	3.721	6.383	
铕标记 Marked by Eu	早稻肖蛸 The spiders Tetragnatha in early rice field	成、亚成蛛 From adult to subadult	4	78.8	6.873	8.749	4.696
空白对照 Control	早稻肖蛸 The spiders Tetragnatha in early rice field	成、亚成蛛 From adult to subadult	4	105.7	3.861	4.053	
铕标记 Marked by Eu	早稻微蛛 The spiders Ummeliata in early rice field	成、亚成蛛 From adult to subadult	6	12.7	4.256	32.906	11.880
空白对照 Control	早稻微蛛 The spiders Ummeliata in early rice field	成、亚成蛛 From adult to subadult	6	20.4	4.253	21.026	
铕标记 Marked by Eu	晚稻狼蛛 The spiders pirata in late rice field	成、亚成蛛 From adult to subadult	4	54.2	4.683	8.755	5.015
空白对照 Control	晚稻狼蛛 The spiders pirata in late rice field	成、亚成蛛 From adult to subadult	7	113	4.197	3.740	
铕标记 Marked by Eu	晚稻肖蛸 The spiders Tetragnatha in late rice field	成、亚成蛛 From adult to subadult	6	151.7	10.776	7.180	3.559
空白对照 Control	晚稻肖蛸 The spiders Tetragnatha in late rice field	成、亚成蛛 From adult to subadult	5	105	3.771	3.621	
铕标记 Marked by Eu	晚稻微蛛 The spiders Ummeliata in late rice field	成、亚成蛛 From adult to subadult	8	24.5	6.530	26.560	12.028
空白对照 Control	晚稻微蛛 The spiders Ummeliata in late rice field	成、亚成蛛 From adult to subadult	6	15.2	2.191	14.532	

蛸、食虫沟瘤蛛平均每天捕食叶蝉的数量分别为 4.63 头、2.81 头、1.0 头（计算方法为：每头蜘蛛平均每天捕食叶蝉的数量＝早、晚稻 100 头蜘蛛的含铕量/早、晚稻 100 头叶蝉的含铕量/早、晚稻 100 头叶蝉的重量/叶蝉被吸食率（65%））。

4　小结与讨论

试验结果表明，所选用的稀土元素铕可以通过施用于泥土被水稻吸收，叶蝉取食水稻，狼蛛、肖蛸、微蛛等蜘蛛捕食叶蝉这一过程而传递，证明该荧光示踪物用于水稻-叶蝉-蜘蛛间食物链营养关系定量分析是可行的，是一种比较理想可靠的新方法，并同样适用于对稻田生态系统其他食物链营养关系的测定。

早稻孕穗期和晚稻分蘖期施铕 7d 后，测定其含铕量均表现为叶片＞茎及叶鞘。根据早、晚稻期叶蝉取食水稻后测定的含铕量和叶蝉体重的不同进行核算，早稻期每 100 头叶蝉每天可取食水稻 1.13g，晚稻期可取食水稻 0.71g。

根据早、晚稻期投放拟水狼蛛、锥腹肖蛸、食虫沟瘤蛛 3d 后测定的含铕量计算，每头拟水狼蛛、锥腹肖蛸、食虫沟瘤蛛平均每天捕食叶蝉分别为 4.63 头、2.81 头、1.0 头。捕食能力：拟水狼蛛＞锥腹肖蛸＞食虫沟瘤蛛。

采用稀土元素铕荧光物示踪定量分析稻田生态系统各食物链的营养关系是一个全新的课题，未知的及影响检测结果的因素很多，有待进一步研究。

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