OBSERVATIONS ON SUGARBEET ROOT PENETRATION BY *HETERODERA BETAE* AND ITS DEVELOPMENT DURING TWO CROP GROWING SEASONS IN ITALY

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Summary. Investigations were undertaken during 2000 and 2001 at two localities in Piedmont to study the life cycle of *Heterodera betae* on sugarbeet. The invasion of roots occurred in both fields at the same mean soil temperature of 8 – 9°C, but the life cycle of *H. betae* differed markedly during the two seasons characterized by different climatic conditions. During the first year at S. Michele the climatic conditions resulted warmer and moister than those recorded at Solero during the second year of sugarbeet culture. In 2000 *H. betae* completed four generations, the reproduction rate was 199 and the yield obtained of only 400 quintals/ha while in 2001 there were only three generations and the reproduction rate was 46 with sugarbeet yield increase of 45%.

The "yellow beet cyst nematode" (YBCN), considered to be a host race of *Heterodera trifolii* Goffart attacking sugarbeet in Europe (Maas and Heijbroek, 1982; Andersson, 1984; Vallotton, 1985; Schlang, 1990; Bossis *et al.*, 1997; Ambrogioni *et al.*, 1999), has recently been described as *Heterodera betae* sp. n. Wouts, Rumpenhorst *et* Sturhan (Wouts e*t al.*, 2001).

Although the host range of *H. betae* is wide and includes species of Cruciferae, Chenopodiaceae, Polygonaceae, Caryophyllaceae and Leguminosae, at present this cyst nematode appears to be economically important only in The Netherlands where damage occurs in sugarbeet fields with an initial soil population of 5 eggs and juveniles/cc soil and in pea and bean fields with over 8 eggs and juveniles/cc soil (Maas and Rothuis, 1988).

This cyst nematode has recently been found in Italy and considered to be a potential pest of sugarbeet (Ambrogioni *et al.*, 2002). As a basis for establishing suitable control measures, investigations were undertaken in 2000 and 2001 in two different localities of Piedmont infested with *H. betae*, in order to provide information on the population dynamics in the soil and sugarbeet roots and the yield losses.

MATERIALS AND METHODS

Two fields, both in the province of Alessandria, were selected for the experiment; in 2000 at S. Michele and in 2001 at Solero. The sugarbeet crops were included in three year rotations with cereals as non host crops.

At the beginning of the growing season the initial field population of *H. betae* Wouts *et al.* was 4.5 eggs and juveniles in cysts per cc of soil at S. Michele and 8.8 at Solero.

In 2000 sugarbeet (*Beta vulgaris* var. *saccarifera*) cv. Zaira was sown on 2 March and in 2001 sugarbeet cv. Dorotea was sown later (13 April) because of previous abundant rainfall.

The normal agronomic practices were applied in both fields.

Root and soil samples from the rhizosphere were collected at random every three days from 10 April 2000 and 27 April 2001 for three and four times respectively and then once a week until the crop harvest. Three g root samples, were washed, fixed and processed by Coolen's method (1979) to recover the different life stages. From a 500 cc soil sample, well mixed, two 100 cc aliquots were processed by the cotton wool filter method (Oostenbrink, 1960) to estimate the numbers of second stage juveniles (JJ2). The remaining 300 cc were air dried and cysts were extracted with the Fenwick can; these cysts were subsequently crushed to determine the number of eggs and JJ2 present.

Soil temperature at 20 cm depth and rainfall were recorded throughout the two field experiments (Fig. 1).

RESULTS

In 2000 (Tables I and II) second stage juveniles (JJ2) were recovered from the roots at eighteen days after plant emergence (23 March). After three days from root penetration 11% of JJ2 appeared swollen and after a week 7% of JJ2 had started moulting to third stage. Third stage juveniles (JJ3) were found after two weeks; fourth stage juveniles (JJ4) and a few white females after three weeks.

Egg sacs without eggs and yellow females with a few non-embryonated eggs were detected one and two weeks later, respectively, whereas newly formed cysts occurred on the roots forty days after root invasion. Many of the females produced enlarged egg sacs which were empty or contained 20-25 eggs at most.

By 5 June the number of JJ2 in the roots had in-

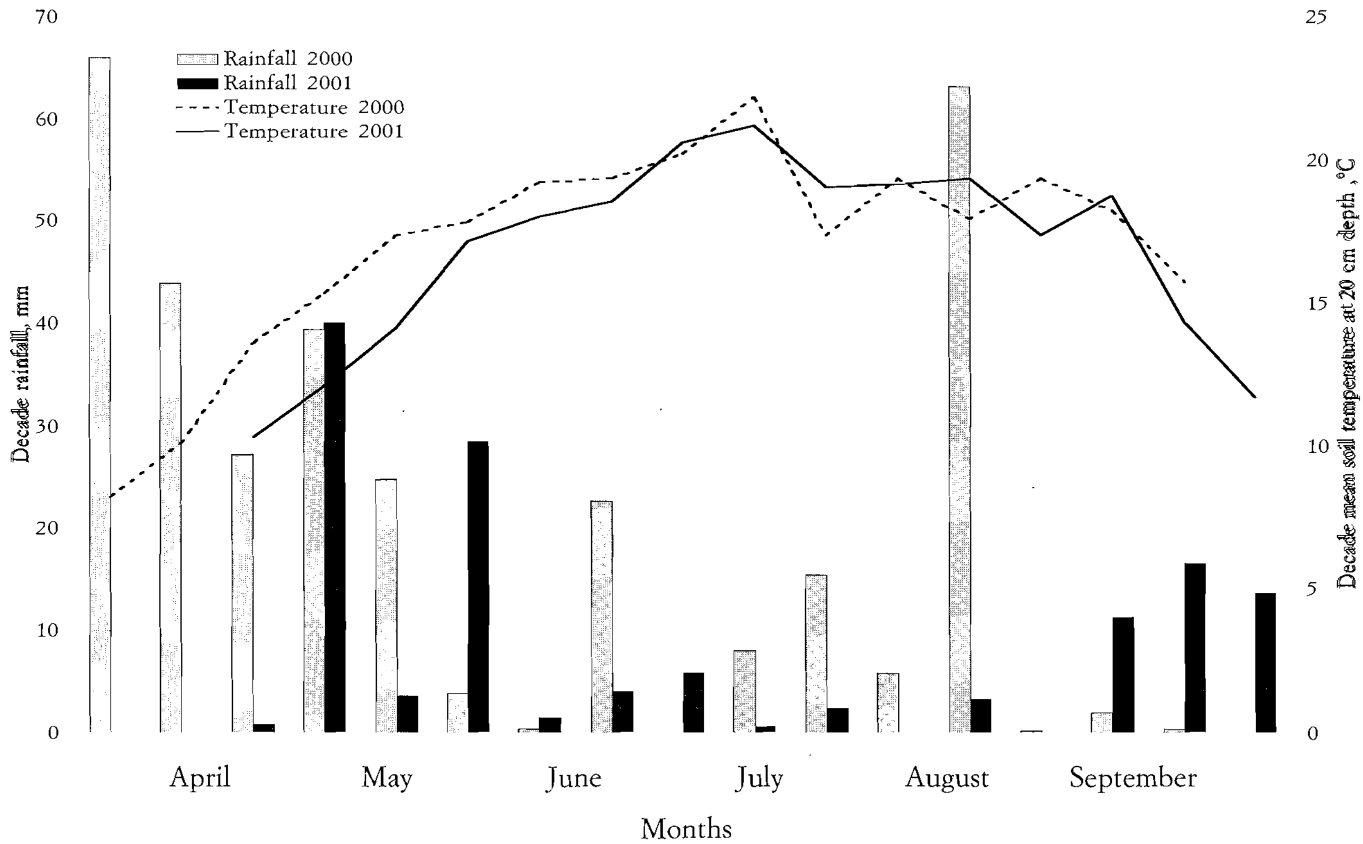

Fig. 1. Field environmental conditions during the *Heterodera betae* development on sugarbeet crops (10 April-11 September 2000; 27 April-18 September 2001).

creased, indicating the second generation had begun. The first generation, calculated from the root penetration time by juveniles to the hatching of JJ2 from the new cysts, was completed in eight weeks at a mean soil temperature of 15 °C (5.2-23.7).

The third generation began on 3 July, the fourth on 31 July and the fifth on 4 September, but the latter one was terminated by crop harvest.

The time required for the second and third generations was the same (four weeks) at an average soil temperature of 19.7 °C (16.4-24.1) and 19.5 °C (14.8-26.0) respectively; the fourth one, at soil mean temperature of 18.2 °C (15.0-20.5), was one week longer.

Since the sixth week from the start of observations and until the crop harvest all stages were always present inside the roots; the maximum number level was observed for JJ2 and JJ3 at seventeen weeks from root penetration; for JJ4, females and cysts at four, thirteen and twenty one weeks respectively. Total number of nematodes in the roots reached a maximum (1315 individuals/g) in early August during the fourth generation.

The dynamics of JJ2 emerged from newly formed cysts of *H. betae*, clearly showed the beginning of each of the five generations, confirming the findings reported

Table I. Number of different stages of *Heterodera betae* recovered from sugar beet roots at S. Michele.

Developmental stages/g root	Sampling date (2000)																							
	10/4	13/4	17/4	24/4	1/5	8/5	15/5	22/5	29/5	5/6	12/6	19/6	26/6	3/7	10/7	17/7	24/7	31/7	7/8	14/8	21/8	28/8	4/9	11/9
JJ2	43	85	105	113	129	92	8	5	5	15	128	105	86	137	214	611	285	602	829	257	134	113	571	65
JJ3				6	86	143	7	4	11	1	3	20	81	69	21	15	209	227	300	117	123	49	34	50
JJ4					57	157	15	9	12	1	3	3	79	38	18	5	16	110	73	30	61	43	27	9
♀♀					2	47	18	43	63	12	3	1	32	210	334	40	30	104	56	34	58	156	93	60
Cysts								6	16	27	17	4	3	16	62	71	49	56	57	24	31	60	230	82
Total number	43	85	105	119	274	439	48	67	107	55	154	133	281	470	649	742	589	1099	1315	462	407	421	955	266

Table II. Soil population densities of *H. betae* in the sugar beet field at S. Michele.

	Sampling date (2000)																							
	10/4	13/4	17/4	24/4	1/5	8/5	15/5	22/5	29/5	5/6	12/6	19/6	26/6	3/7	10/7	17/7	24/7	31/7	7/8	14/8	21/8	28/8	4/9	11/9
JJ2/100 cc soil	120	132	65	81	84	18	13	4	6	197	900	365	244	415	1096	1307	285	6970	5098	1260	1250	1011	3273	5178
Cysts/100 cc soil	22	29	11	16	14	17	7	4	7	21	112	35	98	77	105	262	356	305	768	529	630	1127	904	989
JJ2/cyst	25	25	20	10	1	2	10	10	118	236	225	285	137	244	59	161	206	215	10	9	67	79	156	91
JJ2 cysts/cc soil	5.5	4.5	2.2	1.6	0.2	0.3	0.7	0.4	8.3	49.5	251.9	99.8	134	188	62	422	732	656	80	47	424	887	1414	896

for the roots. JJ2 were always present in the roots or in the soil reaching in this case the maximum number (6790 JJ2/100 cc soil) at the end of July when the fourth generation began. A very high number of JJ2 (5178/100 cc soil) was present also at the harvest, according to the considerable number of cysts accumulated during the growing season (1127 cysts/100 cc soil) at the end of August and to their very high content of eggs and JJ2 (1414/cc of soil) at the beginning of the last generation.

The reproduction rate of *H. betae* population, ratio between final and initial densities of eggs and juveniles in cysts in the soil, was 199.

The yield of sugarbeet in the experimental field was 20% less than values usually recorded in the same area and 43% less than from fields in uninfested areas.

In the second year the root penetration by JJ2 was recorded at just four days after plant emergence (Tables III and IV) (23 April). After three days 43% of them was represented by swollen JJ2 and after six days JJ3 were already present. JJ4 and a few white young females appeared seventeen days after root penetration; during the following week some females were surrounded by a small gelatinous matrix and others passed through the yellow phase. Newly formed cysts appeared on the roots one month after JJ2 invasion. The egg sacs were often empty or containing only up to 10 eggs.

The first generation was completed in seven to eight weeks at a mean soil temperature of 15.5 °C (6.7-25.2). By mid June the increase of JJ2 in the roots indicated the start of the second generation that was completed in four weeks at an average soil temperature of 20.5 °C (12.7-26.8). The occurrence of the third generation began on 16 July and was completed in six weeks at a mean soil temperature of 18.4 °C (13.2-25.8). The fourth generation began on the end of August, but it was terminated by the crop harvest.

During the sugarbeet growing season there was a continuous root penetration by JJ2 which reached the maximum number during the third generation at about fourteen weeks from the former invasion of the roots; always at the same period JJ4 and females were present with their maximum number after thirteen and eleven weeks respectively. The highest number of JJ3 and cysts was present inside the roots during the second generation after ten and seven weeks. Maximum total number of different developmental stages recovered from roots (1004 individuals/g) occurred during the first week of August.

With the data from the second year of the experiment it was established that three generations of *H. betae* were completed and a fourth had commenced. During the third generation, at the end of July, there was in the soil the maximum number either of free JJ2 (6400 JJ2/100 cc) or eggs and JJ2 in cysts (483/cyst). Obviously the number of cysts accumulated in the soil and their content of eggs and JJ2 per cc of soil reached the maximum in September at the end of the sugarbeet crop with values of 478 cysts/100 cc soil and 762.6/cc soil respectively.

The reproduction rate of *H. betae* at Solero was 46 and a yield increase of 45%, compared with the value obtained at S. Michele.

Table III. Number of different stages of *H. betae* recovered from sugar beet roots at Solero.

Developmental stages/g root	Sampling date (2001)																						
	27/4	30/4	3/5	7/5	14/5	21/5	28/5	4/6	11/6	18/6	25/6	2/7	9/7	16/7	23/7	30/7	6/8	13/8	20/8	27/8	3/9	10/9	17/9
JJ2	10	30	84	46	212	24	16	11	1	232	251	483	166	354	449	501	655	479	75	79	174	98	171
JJ3			8	16	101	92	21	8	2	1	30	104	236	127	131	184	104	160	47	55	26	62	56
JJ4					72	122	14	7	7	0	3	83	180	85	12	224	96	43	40	22	5	37	9
♀♀					12	62	61	52	33	50	19	17	128	174	27	31	77	15	73	52	19	37	23
Cysts							2	12	20	107	14	12	11	48	34	35	72	29	21	38	63	37	55
Total number	10	30	92	62	397	300	114	90	63	390	317	699	721	788	653	975	1004	726	256	246	287	271	314

Table IV. Soil population densities of *H. betae* in the sugar beet field at Solero.

	Sampling date (2001)																					
	27/4	30/4	3/5	7/5	14/5	21/5	28/5	4/6	11/6	18/6	25/6	2/7	9/7	16/7	23/7	30/7	6/8	13/8	20/8	27/8	3/9	10/9	17/9
JJ2/100 cc soil	34	51	51	57	124	44	259	16	11	808	2700	3263	1136	2965	6400	3770	1578	1268	355	380	585	1600	493
Cysts/100 cc soil	10	10	10	9	9	20	8	5	2	6.3	18	33	40	101	121	157	152	62	93	217	140	383	478
JJ2/cyst	88.3	75.7	73	54	32.7	5.5	77.5	40.6	17.5	165.5	216.9	174.2	198	483	121.2	223.5	140.2	150	100.5	89	48	199	85.5
JJ2 cysts/cc soil	8.8	7.6	7.3	4.9	3	1.1	6.2	2	0.7	10.4	39.04	57.5	79.2	487.8	147	350.9	213.1	92.7	93.5	193.1	67.2	762.6	408.9

DISCUSSION AND CONCLUSION

The field experiments indicate that the life cycle of the cyst forming nematode *H. betae* is influenced by soil temperature and moisture, both considered determining factors in the rate of development of endoparasitic sedentary females (Jones, 1975).

The two sugarbeet growing seasons differed mainly in lower mean soil temperatures and a drier climate recorded in spring and summer, in 2001, at Solero, compared with similar climatic data in 2000, at S. Michele.

During the two years of the trial *H. betae* completed its first generation in 56 days in 2000 and 52 in 2001 at mean soil temperatures of about 15 °C even though the sugarbeet was drilled at S. Michele about 6 weeks before that at Solero. In spring 2001, JJ2 hatched promptly from the cysts in the soil and immediately invaded the roots; all the developmental stages had shorter life spans than at S. Michele, but the emergence of JJ2 of the second generation was probably delayed by lower mean soil temperatures and drier climate. However, in both locations JJ2 emerged from cysts and penetrated the roots at mean soil temperatures of 8-9 °C, in contrast to an optimum temperature of 15 °C for *H. betae* observed by Maas and Heijbroek (1982).

In both experiments the second generation reached completion in four weeks at an average soil temperature of 20 °C, but the time required by the third generation was six weeks in 2001 and four in 2000. The latter was due not only to the lower mean soil temperature in 2001 compared with 2000 (18.4 °C versus 19.5 °C), but mainly to the lack of rain that reduced the soil moisture and therefore delayed the hatching of JJ2 from the cysts. In fact at Solero three support irrigations were supplied while only two at S. Michele. The fourth generation of *H. betae* in 2001 was terminated at harvest of the crop.

In conclusion, the data revealed some very important differences between the two subsequent sugarbeet cropping seasons.

In the field located at Solero compared with that of S. Michele the delayed sugarbeet sowing, the colder and drier season and also the initial larger field nematode populations (8.8 vs 4.5 eggs and juveniles/cc soil) showed to be determining factors affecting the developmental cycle of *H. betae* with a reduced number of generations (only three), the reproduction rate of 46 and a consequently yield increase of 45%. These factors can play an important role in a rotation with non host crops to limit the heavy yield losses causes by this cyst nematode which can be considered a new important pest for sugarbeet production in Italy.

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