DEPARTMENT OF AGRICULTURE, SOUTH AUSTRALIA

Agronomy Branch Report

PARASITES OF Sitona humeralis Steph. (COLEOPTERA:CURCULIONIDAE)

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Senior Research Officer, Entomology.

Report No. 36

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SUMMARY:

Insecticidal control of the introduced Sitona humeralis Steph. is not feasible in South Australia because of extensive breeding of high densities, because of the actively flying adults and because the larvae occur in soil under established pasture or lucerne crops. There are a number of known parasites of S. humeralis and other Sitona spp. in their countries of origin. Parasites which have been studied and released in North America against S. cylindricollis are described. Releases were not successful but experience gained is useful background information when biological control of S. humeralis by introducing parasites is attempted in southern Australia.

1. BACKGROUND:

The introduced Sitona weevil, Sitona humeralis Steph., was first found in South Australia in 1966 and since then has become established in very high densities over practically all of South Australia's agricultural areas and beyond into some pastoral areas. The distribution, biology, damage and importance of this pest to South Australia is discussed by Allen (1971).

Insecticidal control is not feasible because of extensive breeding of vast numbers and actively flying adults which can reinfest small areas of treated pastures or lucerne crops. With choice of insecticides limited to relatively "short-lived" organic phosphorus compounds, re-infestation is critical. Cost of re-treatment would be uneconomic in grazing situations and could only be considered in limited situations where high value crops, e.g. legume seed crops or legume seedlings, require protection. The soil-dwelling larvae damage legume nodules in established legume-based pastures and lucerne crops. Insecticidal control in these situations is very difficult and generally impractical.

Biological control of sitona weevil with introduced parasites combined with legume tolerance/resistance breeding offers the most rational approach to sitona weevil control in southern Australia.

2. PARASITES OF SITONA SPP.:

Sitona spp. are native to Europe, the Mediterranean, Central Asia and North America. Parasites of Sitona spp. in these areas have been well documented and a list of parasites, their Sitona hosts and country where they were found is shown in Appendix I.

Biology of a number of these parasites was studied prior to their release into America and Canada for control of the sweet-clover weevil, Sitona cylindricollis Fahr. The main parasites were three species of Braconid wasps, Microctonus aethiops (Nees), Perilitus rutilus (Nees), and Pygostolus fulcatus (Nees) and the Tachinid fly, Campogaster exigua (Meig.).

2.1 Distribution & Hosts of Parasites

2.1.1 Braconid wasps

M. aethiops was reared from mass collections of Sitona in France (Berry et al 1950), from S. hispidula in Russia (Meyer 1934), from S. humeralis and S. crinita in France and from S. hispidula in Sweden (Loan et al 1961). The mass collections of Sitona in France were predominantly S. humeralis, with a number of S. lineata and S. puncticollis and some S. sulcifrons, S. hispidula, S. crinita and S. flavescens. M. aethiops was reared from the Chrysomelids, Phyllotreta vittula in Germany (Kaufman 1923) and P. memorum

in England (Newton 1931) and from the Curculionid, Hypera postica in France (Loan et al 1961).

P. rutilus was reared from the same mass collections of Sitona in France as M. aethiops (Berry et al 1950), from S. hispidula and S. lineata in England (Jackson 1922, 1928), from S. lineata and S. hispidula in Sweden and S. humeralis and H. postica in France (Loan et al 1961), and from Sitona hosts in Italy (Parker, USDA). Jackson (1928) summarised the known distribution of P. rutilus in continental Europe and the British Isles.

In France, S. humeralis and H. postica are probably the major host species for both M. aethiops and P. rutilus.

P. falcatus was reared from S. lineatus and S. hispidula in England (Jackson 1922), from S. humeralis, S. crinitus and S. inops (Grossheim 1928, Meyer 1934) and from S. lineatus and S. crinitus (Ulashkevich 1935) in U.S.S.R., and from S. lineatus, S. hispidula and S. humeralis in Sweden (Loan et al 1961a).

2.1.2 Tachinid fly

C. exigua was reared from S. humeralis, S. lineata, S. hispidula and H. postica in northern France and is probably a parasite of all the Sitona spp. mentioned above in the mass collections from France (Berry et al 1950). C. exigua has not been found in Sitona adults collected from southern France.

2.2 Biology of Parasites

2.2.1 Braconid wasps

Biology of M. aethiops and P. rutilus is similar (Loan et al 1961). Mated females produce progeny of both sexes, and parthenogenetic reproduction results in males only. Mating is effected within minutes of emergence and males mate once or twice only and attempt to mate only with virgin or newly mated females. Oviposition usually takes place while the adult host is moving. It is effected through the membranous area at the apex of the abdomen but can be attempted, unsuccessfully, in the face or thoracic regions. The egg is deposited freely in the haemocoele. A parasite may strike many times without succeeding in depositing an egg. Parasitisation occurs during day and night. Female parasites do not distinguish between parasitised and non-parasitised hosts but in the event of superparasitism only one parasite larva

survives. There is no oviposition preference between Sitona spp. and some Hypera spp. There was no data on total number of eggs laid per female parasite, but two females of P. rutilus laid 45 and 50 eggs, respectively, in 11 days when they were supplied with ten weevils each at successive 24 hour intervals. Fully developed fifth instar larvae drop free of the weevil host and immediately burrow between particles of soil and spin cocoons from which the adults emerge after one to two weeks, depending on the conditions and species of the parasite.

Longevity of M. aethiops females ranges from 6 to 17 days (mean 12.1 days) at 74°F and under cooler insectary conditions the time varies from 11 to 22 days (mean 16.9 days). Males are shorter lived. Longevity of mated P. rutilus females varies from 24 hours to 17 days in the insectary and at 74°F males and females live about two weeks. Approximate periods of development for M. aethiops at 74°F are egg 4 to 5 days, larval stages 8 to 9 days, and pupa 8 to 9 days; periods for P. rutilus at 74°F are egg 6 to 7 days, larval stages 9 to 12 days, and pupa 9 to 14 days. Both species have five larval instars.

Biology of P. falcatus described by Loan et al (1961a) is similar to the above Braconids in method of parasitisation and number of larval instars. The fifth larval instar emerges from the host and spins a cocoon on foliage or other places in the weevil habitat (Loan 1961). Reproduction in P. falcatus is parthenogenetic and thelyotokous. Maximum number of eggs laid by an individual female was 46. Longevity of P. falcatus females ranges from 5 to 15 days (mean 11.0 days) at 74°F and under field conditions they live from 7 to 11 days (mean 9.4 days). Periods of development for P. falcatus would probably be similar to the above two Braconids.

2.2.2 Tachinid fly

Little was known about C. exigua (Berry et al 1950) and later references were not found. Reproduction is bisexual and viviparous. Females mate and after a gestation period of 10 to 15 days begin larviposition. (During the gestation period they are fed sugar and honey water in the laboratory). Warm sunlight and high humidity are most favourable for larviposition. Apparently females deposit larvae on the outside of the host between the head and thorax or between the first and second thoracic segments. Deposition is completed so quickly, and the fly breaks away so rapidly, that it does not seem possible that an ovipositor could have been inserted into the body of the host in the short time.

On completion of the first instar or early in the second instar, larvae become fixed into tracheae near either the thoracic spiracle or a spiracle of the anterior abdominal segment. They then form respiratory sheaths in which the posterior half of the body is retained until completion of the larval period. Final larval instars pupate in the host's body. Longevity of adults is from 20 to 30 days at laboratory temperatures. Larval stage in the host is 20 to 30 days and the pupal stage is 18 to 25 days, or a minimum of 48 to 70 days for a complete generation.

2.3 Seasonal History of Parasites

In France, the three Braconid parasites have a similar seasonal history (Loan et al 1961, 1961a). They overwinter as first instar larvae in weevils which became adult in the previous summer. During spring, larvae develop and emerge as adults by late spring-early summer and parasitise over-wintered weevils. This generation of non-diapausing parasites in old weevils takes approximately $3\frac{1}{2}$ to $4\frac{1}{2}$ weeks to develop from egg to adult, depending on the species. Adults from the mid-summer or second generation parasitise the summer or newly-emerged weevils and the first instar parasite larvae go into dispause for the rest of the season and through winter. Some over-wintered Sitona adults, alive when adults of the mid-summer generation of parasite emerge, may be parasitised. Parasites in these hosts die as diapausing first instar larvae because of the natural mortality of the host within a short period.

Seasonal history of the Tachinid fly is not well understood (Berry et al 1950). Adults emerge from over-wintering puparia in the host. Larvae also probably over-winter in live host beetles, but this has not been definitely proved, though duration of the larval stage is prolonged indefinitely during cold weather.

2.4 Effects of Parasitism

Effect of the three Braconid parasites on adult weevils is similar (Loan et al 1961, 1961a). First, second and third instar larvae are primarily haemophagous and have no effect on fat body in the weevil. Fourth instar larvae consume fat body and the remainder of the haemolymph. Fifth instar larvae emerge from the weevil through the intersegmental membrane between the fifth and sixth tergites. The weevile is active up to and during emergence of the parasite larvae, but after emergence the movements of the weevil are lethargic, one or more legs may be paralysed and it does not feed. Death of host occurs 3 to 24 hours after parasite emergence.

Female weevil's reproductive system is eliminated by the effects of parasitism. The terminal chamber of each ovariole becomes distended and pale yellow in colour at its apex and is reduced in legnth. Eggs in the oviduct also become pale yellow and shrunken, with the yolk mass separated from the chorion. Cessation of oviposition and degeneration of ovaries and eggs within the oviduct are brought about by the egg stage of the parasite. Oviposition of field collected S. cylindricollis stopped within one or two days after deposition of the parasite egg. Oviposition of both overwintered and summer emerged Sitona is prevented. Parasitism does not affect the potency of male weevils.

Effect of Parasitism by C. exigua on Sitona spp. was not evident in the references reviewed.

2.5 Multiparasitism

Since female parasites can not distinguish parasitised from non-parasitised host adult weevils, multiparasitism can occur when more than one parasite is present. Interaction between first instar larvae of P. rutilus and P. falcatus in S. cylindricollis resulted in P. rutilus surviving in 93.1% of the infested weevils.

2.6 Extent of Parasitism

In field populations in France, June, 1957, 5% S. humeralis and 16% H. postica were parasitised by M. aethiops (and, possibly P. rutilus as first instar larvae are identical). This difference may be associated with greater abundance of H. postica in June. In July when S. humeralis was abundant, incidence of parasitism of both species was 5%. In Sweden, parasitism of Sitona spp. with P. rutilus has varied from 4.0 to 12.2%. In laboratory cages, M. aethiops parasitised 56.5% of S. cylindricollis and 26.9% of Hypera meles populations, respectively, and P. rutilus parasitised 26.0% of the weevil population (Loan et al 1961).

Maximum measured number of Sitona weevils parasitised by P. falcatus in field cages was 35.4%; in the laboratory, 31% of weevils were parasitised. In Sweden, a 73.3% parasitism of weevils by P. falcatus in 1958 developed from an overwintering larval population in less than 1% of the weevils (Loan et al 1961a).

In France, 10 to 50% of Sitona were parasitised by C. exigua in the field (Berry et al 1950).

3. SITONA PARASITE RELEASE PROGRAMMES:

The first introduction and release of Sitona parasites was the release of M. aethiops and C. exigua in North Dakota in 1948 (Monro et al 1948). From 1948 to 1958 M. aethiops was introduced into eastern United States against S. cylindricollis and since 1958 all introductions of M. aethiops have been against H. postica (Loan et al 1963, Stehr et al 1971). From 1951 to 1954 releases of M. aethiops and C. exigua collected in France were made against S. cylindricollis in Manitoba, Canada (Loan 1961). Following the failure of establishment of these French parasites, P. rutilus and P. falcatus, collected in Sweden, which has closer climatic similarities with Canada than France, were released in 1958 and 1959. (Loan 1961, Turnbull et al 1961).

3.1 Methods Used for Introduction & Assessment of Sitona Parasites Manitoba, Canada (Loan 1961)

M. aethiops and C. exigua were collected in France. M. aethiops was shipped as larvae or pupae in cocoons while C. exigua was collected in the field and shipped as adults with resting sites and food (agar/honey mixtures). There were few mortalities of M. aethiops but high mortalities of C. exigua. Both parasites were released as adults in Manitoba. From May, 1952 to May, 1954, 889 male and 1,205 female M. aethiops and 12 male and 64 female C. exigua were released.

P. falcatus and P. rutilus were reared from adult S. lineata, S. humeralis, S. hispidula collected from Sweden in June and July from 1956 to 1959. Parasitised adult weevils were shipped to Manitoba in containers with loose foliage of lucerne or vetch. Weevils were then kept in cages with fine mesh floors; final instar larvae of P. rutilus crawled through the mesh floor of cages to form cocoons in soil or on black cloth and P. falcatus larvae formed cocoons on the walls. As adult parasites emerged from the cocoons, they were placed in small wooden cages with honey and water. Without honey, P. falcatus died within one or two days.

Adult S. cylindricollis collected from the field in Manitoba were exposed to the adult parasites and both P. falcatus and P. rutilus were released as first instar larvae in summer emerged weevils. For parasitisation, weevils were exposed in lots of 100-400 to 25-50 parasites for 12-24 hours. 5,000-7,000 weevils were parasitised by P. falcatus out of 18,200 weevils exposed and 750-1,000 were parasitised by P. rutilus out of 5,000 weevils exposed.

Main requirements for release sites were a first year sweet-clover stand to provide food, over-wintering sites for released weevils and a large, natural population of the host weevil, S. cylindricollis. P. falcatus and P. rutilus were released in 1958, but there were poor stands of sweet-clover due to weather conditions and a number of release paddocks were tilled in spring, 1958.

Parasite establishment was assessed by dissection and rearing parasites from weevils collected from the field. In 1952 and 1953, small numbers of weevils were collected from M. aethiops and C. exigua release sites and dissected; in 1954, 6,000 over-wintered and 15,000 summer emerged weevils were collected and held for parasite emergence. In 1959, 90,000 over-wintered weevils and 75,000 summer emerged weevils were collected from the 1958 P. falcatus and P. rutilus release sites. Weevils were either dissected or kept for parasite emergence. In 1960, 3,120 overwintered weevils were collected from or near release sites.

North Dakota, United States (Munro et al 1948)

A few small shipments of M. aethiops and C. exigua adults from France were released during the spring and summer of 1948 in field cages supplied with large numbers of adult S. cylindricollis to be parasitised. When parasites were being collected it was observed that 20-50% of the weevils were "destroyed" by C. exigua and 4% were "destroyed" by M. aethiops.

3.2 Results & Comments on Introductions of Sitona Parasites Manitoba, Canada (Loan 1961)

No larvae of M. aethiops and C. exigua were reared or dissected from field collected S. cylindricollis. Small numbers of C. exigua released may have partly caused failure of C. exigua establishment. The main reason why M. aethiops was not successful was probably because S. cylindricollis was not a suitable host for the strain of M. aethiops collected from France (Turnbull et al 1961). In laboratory tests, over 80% of parasite larvae died shortly after hatching in S. cylindricollis, though they developed normally in other Sitona spp. Loan et al (1961) found that M. aethiops collected from France was a different biological strain from M. aethiops collected from Sweden.

No larvae of P. rutilus were reared or dissected from field collected S. cylindricollis, but P. falcatus was recovered in 1959 and 1960 from weevils collected from one re-

lease site and was considered to be established in the area. In weevil collections made on 15th June, 1959, 24 larvae emerged from 4,500 hosts and 6 larvae were dissected from 520 hosts. From a second collection on June 26th, 7,000 weevils yielded no parasites, but 6 larvae emerged from 10,000 weevils collected a week later. In 1960, 3 larvae emerged from 2,302 weevils swept from a crop 600 yards from a 1958 release site.

P. falcatus was the most successful of the four parasites in these releases but it did not offer economic control of S. cylindricollis. Establishment of P. falcatus was probably least expected since it does not parasitise S. cylindricollis in Europe.

North Dakota, United States

The only information obtained on results of these releases was that recoveries had been made in the season of release, but it was not known whether M. aethiops or C. exigua were permanently established (Claussen 1956).

Further information on M. aethiops introductions

Since 1958, all M. aethiops releases in eastern United States were against Hypera postica, the alfalfa weevil. M. aethiops has been established in this area since 1961 (recovery samples in 1961 yielded 163 parasites from 275 hosts (Loan et al 1963) and by 1970 M. aethiops was established in 11 states (Stehr et al 1971). M. aethiops now appears to be one of the most important parasites in the successful biological control of H. postica in eastern United States (Day et al 1971). M. aethiops is ideal for H. postica control since there are two generations per year, it overwinters as first stage larvae in adult weevils, and hence is well synchronised with weevil activity and disperses with the weevils. From experience, Stehr et al (1971) concluded that the best release site for M. aethiops adults is a dense stand of clean lucerne, not cut, sprayed or disturbed. This results in minimal dispersal of parasites and produces greatest number of weevils for parasitisation. Releases of 1,200 adults (600 females) seems adequate to effect establishment, and although fewer could be released, it is felt that it is preferable to release large numbers of parasites at few sites. Once established, the parasite can be subcolonised in subsequent years at other sites by moving parasitised adult weevils.

4. DISCUSSION:

P. falcatus was the only parasite to become established of all parasites introduced into Canada and the United States to control S. cylindricollis. Although it became established, its control value was limited (McLeod 1962). Reasons for failure of establishment of other parasite species were not described, though it was suggested that non-establishment of M. aethiops and C. exigua from France in Canada may have been due to different climatic conditions between the two countries and that the strain of S. cylindricollis in Canada was not a suitable host for the strain of M. aethiops introduced from France.

M. aethiops, initially released to control S. cylindricollis and not successful, was subsequently released in eastern and mid-west United States against H. postica. It became established and is now one of the main parasites in a successful control programme against H. postica. One reason for the success of M. aethiops against H. postica was the survival of overwintered H. postica adults into the summer to host the mid-summer, second generation of parasites. Survival of overwintered adults into summer also occurs with Sitona spp. in Europe. In Southern Australia, it appears that most overwintered S. humeralis adults have died by late October-early November; the new generation of adults begin emerging in late October and aestivate through the mid-summer months. Hence, the seasonal history of S. humeralis in southern Australia may not suit M. aethiops and also P. rutilus, which has similar requirements to M. aethiops. More information is required on the longevity and rate of decline of overwintered S. humeralis adults in southern Australia.

C. exigua was considered the most promising parasite of Sitona spp. found in France for introduction into northern America to control S. cylindricollis, and, although it did not appear to control Sitona spp. in France, the fact that at times it attacked a high percentage of beetles (up to 50%) indicated that it could have a high potential and may become a controlling factor under some conditions. More information should be obtained on the biology and parasitism of this species to consider whether it would be feasible for introduction into southern Australia. Climatic conditions in southern Australia would be more similar to France than was the case with Canada, the only country where attempts to introduce C. exigua have been made.

Areas in Europe surveyed for parasites of Sitona spp. for introduction into North America and Canada ranged from southern France northwards but exact locations of collection

areas were not published. Initial surveys for parasites to introduce into southern Australia should be made in the Mediterranean Basin in areas with similar climatic conditions to southern Australia; emphasis should be placed on southern Mediterranean areas (Morocco-Algeria-Tunisia) because the morphological form of S. humeralis introduced into Australia closely resembled Algerian material (Chadwick 1960). Information was not available on parasites of Sitona spp. in southern Mediterranean areas.

Biology of all parasites listed in Appendix I was not available, but some of these would not be suitable for introduction against S. humeralis in southern Australia because they were either host specific (Sitona spp. other than S. humeralis) or were found in areas with vastly different climatic conditions. Some of the parasites studied attacked a wide range of hosts, in some cases not even restricted to a single family, and if these were considered for introduction, the full host range and consequent implications would have to be understood.

Although releases of Sitona parasites were not successful in North America against S. cylindricollis, experience gained from this work is good background information and indicates the necessity for a detailed study of the biology of S. humeralis in southern Australia and a survey for parasites in areas in Europe and the southern Mediterranean climatically similar to southern Australia. Also, it is necessary to ensure that the strain of particular parasite(s) introduced will parasitise and survive on the strain of S. humeralis found in southern Australia.

It is still considered that the introduction of parasites is the most feasible approach to S. humeralis control in southern Australia. Sankaran (1970) considered that "European parasites of Sitona and Hypera are worth serious consideration for introduction against S. humeralis in Victoria. Some of these occur in various parts of Europe and Central Asia under different ecological conditions and the chances of obtaining material from suitable areas and establishment in Victoria would appear to be very promising."

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APPENDIX I

List of known parasites of Sitona spp. (after Sankaran 1970)

Parasite	Host Sitona spp.	Country
1. HYMENOPTERA		
Braconidae		
Microctonus aethiops (Nees)	*S. crinita*, *S. humeralis*, *S. lineata*, *S. hispidula*, *S. puncticollis*, *S. sulcifrons*, *S. flavescens*,	France
	S. hispidula	Russia Sweden Britain
M. colesi	*S. cylindricollis*	Canada
M. sitonae Mas.	*S. scissifrons*	Canada
Perilitus rutilus (Nees)	*S. crinita*, *S. humeralis*, *S. lineata*, *S. hispidula*, *S. puncticollis*, *S. sulcifrons*, *S. flavescens*	France
	S. hispidula, *S. lineata*	Britain Sweden
	S. lineata	Germany
	Sitona sp.	Italy
P. cerealium Hal.	*S. sulcifrons*	Britain
P. labilis Ruth.	*Sitona* sp., *S. callosa*, *S. lineata*	Russia
	S. lineata	Germany
Perilitus sp. (or spp.)	*S. callosa* *S. lineata*	Britain Germany
Leiophron lituratus Hal. (= *Centistes excrucians*)	*Sitona* sp.	Russia
	S. scissifrons	Europe America Canada

Appendix I (Contd.)

Parasite	Host Sitona spp.	Country
L. muricatus Hal. var. *nigra*	*S. lineata*	Britain Germany
Leiophron sp. (or spp.)	*S. hispidula* *S. lineata*	Britain Germany
Pygostolus falcatus (Nees)	*S. lineata*, *S. hispidula*, *S. humeralis*, *S. inops*, *S. crinitus*, *S. lineata*, *S. callosa*	France
	S. lineata, *S. hispidula* *S. humeralis*	Sweden
	S. callosa, *S. humeralis*, *S. inops*, *S. lineata*	Germany
Mymaridae *Anaphes* sp.	*Sitona* sp., *S. callosa*, *S. hispidula*, *S. humeralis* *S. inops*, *S. lineata*	Russia
2. DIPTERA		
Tachinidae		
Campogaster exigua (Meig.)	*S. humeralis*, *S. lineata*, *S. hispidula*	France