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REVIEW ARTICLE

Associative nitrogen fixation, C_4 photosynthesis, and the evolution of spittlebugs (Hemiptera: Cercopidae) as major pests of neotropical sugarcane and forage grasses

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Abstract

Neotropical grass-feeding spittlebugs of several genera are important pests of pasture grasses from the southeastern USA to northern Argentina, and of sugarcane from southern Mexico to southern Brazil, causing estimated reductions of up to 70% in yield and estimated monetary losses of US $840–2100 million annually. With few exceptions, the species badly damaged by these spittlebugs are introduced C_4 grasses that exhibit associative nitrogen fixation. This study synthesizes evidence that the pest status of many tropical and subtropical grass-feeding spittlebugs is linked to associative N-fixation in their C_4 hosts. Recognition that associative N-fixation is a major factor in spittlebug host preferences should deepen understanding of spittlebug agricultural ecology and facilitate efforts to combat spittlebug pests. In particular, spittlebugs should be susceptible to manipulation of xylem transport solutes. However, reduction of nitrate fertilizer rates, increase in ammonium fertilizer rates, or enhancement of associative N-fixation as a consequence of genetic engineering could make hosts more susceptible to spittlebug attack. Because of their predilection for C_4 grasses, spittlebugs present a clear counterexample to the hypothesis that herbivores prefer C_3 plants to C_4 plants. Finally, it appears that declines in atmospheric carbon dioxide levels during recent geological history promoted the proliferation of C_4 grasses. This, compounded by human agricultural activities, has driven an ecological and evolutionary radiation of grass-feeding spittlebugs that presents continuing opportunities for the evolution of spittlebug pests.

Introduction

Most plant-sucking insects feed on phloem sap or the fluid contents of individual cells. Spittlebugs (or froghoppers), cicadas, and leafhoppers of the subfamily Cicadellinae feed on xylem sap, a food source 100 to 1000 times more dilute than phloem sap and energetically more expensive to extract (Raven, 1983). Consequently, spittlebugs (Thompson, 1994, 1999), and perhaps cicadas (Young, 1984), often exhibit a preference for nitrogen-fixing plants, which, on average, probably carry a more concentrated and reliable supply of organic nutrients in the xylem sap than their non-fixing counterparts. Neotropical grass-feeding spittlebugs

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(Hemiptera: Cercopidae) are serious pests of many grasses, including sugarcane. Examined closely, their pest status appears to reflect an association between xylem feeding on the part of the bugs and a propensity for N-fixation on the part of their host grasses.

Nitrogen-fixing spittlebug host plants fall into three broad categories, which can be abbreviated as the rhizobial, actinorhizal and associative N-fixing systems (Thompson, 1994). Rhizobial and actinorhizal host plants, confined to the Fabaceae and a taxonomically scattered set of woody plants in the Betulaceae, Casuarinaceae, Coriariaceae, Datiscaceae, Elaeagnaceae, Myricaceae, Rhamnaceae, Rosaceae and Ulmaceae, possess highly evolved symbioses with N-fixing prokaryotes housed in specialized root nodules (Baker & Schwintzer, 1990; Thompson, 1994). Associative N-fixing host plants have prokaryote symbionts that inhabit the host plant rhizosphere or live within host plant tissues as endophytes, but without specialized host structures (Baldani *et al.*, 1997; Reis *et al.*, 2000). Associative N-fixation, which is less efficient than rhizobial and actinorhizal N-fixation, filling only a portion of a host plant's N requirement, is well demonstrated only in rice and several C_4 grasses, including sugarcane and important forage grasses, such as *Brachiaria decumbens* Stapf and *Brachiaria humidicola* (Rendle) Schweick., all of the family Poaceae.

C_4 plants are named for unique 4-carbon photosynthetic metabolic intermediates, which, along with specialized leaf anatomy, enable C_4 plants to concentrate intracellular carbon dioxide at levels far exceeding atmospheric concentrations. This permits plants with C_4 photosynthesis to circumvent important limitations of ordinary C_3 photosynthesis. RuBisCo, the critical photosynthetic enzyme in both the C_3 and C_4 systems, is profoundly inefficient at binding its principle substrate, carbon dioxide (Mann, 1999; Sage, 1999). At ambient carbon dioxide levels, when temperatures exceed about 25°C, plants with C_3 photosynthesis metabolize more carbohydrate through photorespiration than they produce through photosynthesis. In contrast, C_4 plants exhibit little photorespiration (Collatz *et al.*, 1998; Miyao, 2003). With adequate water, they can maximize the advantages of full sunlight and high temperatures, giving C_4 plants a competitive edge and higher productivity in warm, sunny habitats. But, the concentration of carbon dioxide required to make this system work entails extra energy costs, so that at lower temperatures and lower light levels C_3 plants maintain a competitive advantage.

Most C_4 plants are grasses and sedges. Consistent with temperature relationships, C_4 grasses are dominant in the tropics and diminish as a proportion of grassland ground cover toward higher latitudes. With the notable exceptions of rice and bamboo, all cultivated tropical grasses have C_4 photosynthesis, which, in turn, is often linked with associative N-fixation, probably because plants with high photosynthetic efficiency can most easily bear the metabolic cost of feeding rhizospheric or endophytic associates (Heckathorn *et al.*, 1999).

This study examines the hypothesis that the pest status of many tropical and subtropical grass-feeding spittlebugs is closely linked to a capacity for associative N-fixation in their C_4 host grasses. More particularly, it is proposed that N-fixation makes host grasses attractive to spittlebugs and supports spittlebug growth and reproduction particularly well, leading to higher population densities and serious economic damage. A review of the literature demonstrates pervasive associations between pest spittlebugs and associative N-fixing economic grasses, a relationship confirmed by several examples of unusual N-fixing host grasses outside the agricultural arena.

Spittlebugs are major pests: the economic impact of spittlebugs on sugarcane and pastures grasses in the Neotropics

Improved pastures constitute the single largest agricultural land use in the American tropics. Virtually all of these pastures are planted with introduced Old World C_4 grasses, most originating in Africa. The 'Africanization' of Neotropical grasslands began soon after European colonization and still continues, driven by the superiority of the introduced grasses for forage (Parsons, 1970; Smith *et al.*, 1995). In just the last few decades, 50–70 million hectares of native savannas have been planted with recently introduced African grasses of the genus *Brachiaria* (Boddey *et al.*, 1996). Region-wide, spittlebugs of the genera *Aeneolamia, Deois, Mahanarva, Prosapia, Notozulia* and *Zulia* are the most important pasture pests (Smith *et al.*, 1995; Valério *et al.*, 1996, 2001; Peck, 1999). The single most widely planted African forage grass, *Brachiaria decumbens* cv. Basilisk, is particularly susceptible to spittlebug attack (Ferrufino & Lapointe, 1989).

Table 1 summarizes quantitative estimates of spittlebug damage to pasture, sugarcane and maize. Though precise economic impact is difficult to measure (Valério *et al.*, 2001), Nilakhe *et al.* (1985) put Brazilian pasture forage losses to spittlebugs at 15%. Simulation of Colombian pasture dynamics based on Brazilian experimental data predicts decreases of 8–34% in meat and milk production, and 17–69% in net income, assuming intermediate levels of spittlebug infestation. This corresponds to projected direct cash losses to Colombian producers of US $40–143 million year^{-1} (Holmann & Peck, 2002). In addition, adult pasture spittlebugs sometimes cause severe damage to adjoining grain crops. The Brazilian State of Mato Grosso do Sul, for example, lost 23,000 ha of rice to spittlebug damage in a single growing season (Nilakhe, 1985).

Spittlebugs of the same genera, and, in many cases, the same species, are also important pests of sugarcane from southern Mexico to southern Brazil (Fewkes, 1969; Valério *et al.*, 2001). In some localities, including Trinidad and parts of Mexico, Central America and Brazil, they are the most important sugarcane pests. Historically, Trinidad plantations have been particularly hard hit by the spittlebug *Aeneolamia varia* (Fabricius), which causes estimated yield reductions of up to 30%, for annual losses of US $4.76 million year^{-1} (CAB International, 2001). In the Ribeirão Preto region of São Paulo State, Brazil, the spittlebug *Mahanarva fimbriolata* (Stål) has recently emerged as a major pest, causing production losses of up to 70% in affected plantations (Dinardo-Miranda *et al.*, 1999; Marin-Morales *et al.*, 2002).

No one has attempted precise global damage estimates for grass-feeding spittlebugs. However, very rough estimates are possible for pasture grasses, assuming that the Holmann-Peck model for Colombia (see above) is applicable to the rest of the Neotropics. Colombia has approximately 5.7 million ha planted in spittlebug susceptible pasture grasses, with cash losses of about US $7–25 ha^{-1} (based on

Table 1. Estimated yield reduction/economic loss attributable to damage by grass-feeding spittlebugs.

Crop	Geographic region	Yield reduction/economic loss estimate	Primary spittlebug pest genera	Reference
Sugarcane	Brazil	30–60% reduction in cane yield	*Mahanarva*	Dinardo-Miranda *et al.*, 1999
Sugarcane	Brazil	Up to 70% reduction in cane yield	*Mahanarva*	Marin-Morales *et al.*, 2002
Sugarcane	Trinidad	30% reduction in cane yield, loss of US $4.8 million year^{-1}	*Aeneolamia*	CAB International, 2001
Sugarcane	Trinidad	30–45% reduction in cane yield	*Aeneolamia*	Fewkes *et al.*, 1965
Sugarcane	Guatemala	14–24% reduction in sugar ton^{-1} of cane yield	*Aeneolamia*	Díaz, 1981
Sugarcane	Guatemala	Reduction in yield, cane 10 t ha^{-1}, sugar 12.8 kg ton^{-1}	*Aeneolamia*	Salguero-Navas *et al.*, 2000
Sugarcane	Guatemala	Reduction in sugar yield, 3.2 kg ton^{-1} of cane/adult spittlebug	*Aeneolamia*	Salguero-Navas *et al.*, 2000
Sugarcane	Venezuela	Up to 27% reduction in cane and sugar yield	*Aeneolamia*	Salguero-Navas *et al.*, 2000
Cattle pasture	Colombia	17–69% loss in net income (US $40–143 million year^{-1})	*Aeneolamia*, *Zulia*	Holman & Peck, 2002
Cattle pasture	Brazil	15% reduction in forage yield	*Deois*, *Notozulia*	Nilake *et al.*, 1985
Maize	China	Up to 50% reduction in yield	*Callitettix*	Lie *et al.*, 1992
Sugar and pasture	Neotropics	Losses of US $840–2100 million year^{-1}	Multiple	See text for derivation

Includes only quantitative estimates.

data in Holmann & Peck, 2002). Regionally, spittlebug susceptible grasses total about 70 million ha (taking the high end of the estimate of Boddey *et al.*, 1996, for *Brachiaria* spp. to account for other susceptible grasses). Scaling up, this corresponds to estimated regional losses of US $490–1750 million, omitting indirect costs, such as long-term pasture degradation (Smith *et al.*, 1995). Losses in sugarcane are even harder to estimate, but given annual production in Mexico, Central America, Trinidad and South America of about 38 million metric tons, a price of US $175 t^{-1}, and a 5% average reduction in sugar production attributable to spittlebugs, the loss would equal 2 million t year^{-1} or US $350 million. Therefore, a crude estimate of world losses to grass feeding spittlebugs is about US $840–2100 million year-1, excluding damage in Africa and Asia, for which no estimates are possible. More exact estimates will require more extensive data on losses by crop and region.

Spittlebug pest host grasses exhibit a capacity for associative N-fixation

Table 2 summarizes evidence for significant associative N-fixation in host grasses of spittlebug pests worldwide. It omits cases in which measured levels of associative nitrogen fixation are less than 5 kg ha^{-1} year^{-1} and those in which the spittlebug host status of the grass is minor, incidental or non-economic (in two cases, *Pennisetum americanum* (L.) Leeke and *P. clasdestinium* Hochst. Ex Chiov., the evidence for substantial fixation is strong but not securely quantifiable). Quantitative estimates of N-fixation must be interpreted with caution, for reasons discussed below.

Table 2 has several notable features. Firstly, with the exception of *Brachiaria brizantha* (Hochst. Ex A. Rich.) Stapf, it includes virtually all forage grasses of major consequence in the New World tropics, as well as sugarcane, rice, sorghum, maize and millet. Almost all economically important grasses with associative N-fixation attract spittlebug pests, especially, but not exclusively, in the warmer regions of the Americas. Secondly, with the exception of rice, all associative N-fixing spittlebug hosts are C_4 grasses. Thirdly, with the exception of *Zea mays* L., all of the New World pest host grasses originate in the Old World tropics. Spittlebug pests attack associative N-fixing grasses in every major tropical and subtropical area, but inflict their worst damage on Old World grasses cultivated in the Americas. In every case, the spittlebugs are native to the region in question.

Spittlebugs also damage five cultivated tropical C_4 grasses that have not been tested for N-fixation (table 3). The patterns established above suggest that many or all of these species may prove to exhibit associative N-fixation.

In at least six additional cases (table 4), N-fixing C_4 forage grasses attract spittlebugs, but the spittlebugs have not been reported to do serious damage. Two of these cases involve native New World grasses (*Axonopus compressus* (Swartz) Beauv. and *Paspalum notatum* Flugge), suggesting that these species evolved tolerance to native spittlebugs long before they were propagated for forage. *Andropogon gayanus* Kunth, an African grass, has natural resistance to spittlebugs (Valério *et al.*, 2001), perhaps a result of tough sclerenchyma tissue surrounding the peripheral vascular tissue (Pires *et al.*, 2000a). *Sorghum halepense* L. more often occurs as a weed than a purposefully planted forage grass and has marginal levels of reported N-fixation (table 4). *Rhynchelytrum repens* (Willd.) C.E. Hubb. is an annual or short-lived perennial that is not a major forage source in the Americas (Judd, 1979). *Brachiaria brizantha* cv. Marandu has antibiotic resistance to certain spittlebugs (Ferrufino & Lapointe, 1989) and has been widely planted throughout the American tropics (Miles *et al.*, 1995; Smith *et al.*, 1995). However, there are recent reports that cv. Marandu is susceptible to damage by spittlebugs of the genera *Zulia* and *Mahanarva* (CIAT, 2000; Valério *et al.*, 2001) and stricter requirements for soil fertility and drainage limit its use as an alternative to spittlebug-susceptible *B. decumbens* (Keller-Grein *et al.*, 1996).

Table 2. Nitrogen-fixing grasses (Poaceae) with spittlebug pests.

N-fixing host grass species	Host geo-origin	Evidence for N-fixation	Quantitiative estimate of N-fixation	Selected N-fixation reference	Primary spittlebug pest genera*	Spittlebug pest geolocalities	Selected spittlebug pest references
Brachiaria decumbens Stapf	AF	$^{15}N_2$	6–20% total N	Reis *et al.*, 2001	*Aeneolamia, Deois, Notozulia, Prosapia*	SA	Linares & Pérez, 1985; Lapointe *et al.*, 1992
Brachiaria humidicola (Rendle) Schw.	AF	$^{15}N_2$	8–20% total N	Reis *et al.*, 2001	*Deois, Notozulia*	CA, SA	Lapointe *et al.*, 1992; Valerio *et al.*, 1996
Brachiaria mutica (Forssk.) Stapf	AF	C_2H_2	9 kg ha^{-1} year^{-1}	Maasdorp, 1987	*Aeneolamia, Prosapia*	CA, CB, SA	Coronado & Sosa, 1966; Linares & Pérez, 1985
Brachiaria ruziziensis Germain & Evrard	AF	$^{15}N_2$	18–23% total N	Reis *et al.*, 2001	*Deois, Notozulia*	SA	Keller-Grein *et al.*, 1996
Cenchrus ciliaris L.	AF, AS	$^{15}N_2$	6–11 kg ha^{-1} year^{-1}	Morris *et al.*, 1985	*Aeneolamia, Clovia, Notozulia, Prosapia*	AS, CA, SA	Botelho *et al.*, 1985; Martin *et al.*, 1995
Cynodon dactylon (L.) Pers.	AF	$^{15}N_2$	2–19 kg ha^{-1} year^{-1}	Morris *et al.*, 1985	*Clovia, Prosapia*	AS, CB, US	Taliaferro *et a.*, 1967; Barrientos *et al.*, 1988
Cynodon nlemfuensis Vanderyst	AF	N-increase	300 kg ha^{-1} year^{-1}	Dart, 1986	*Aeneolamia, Prosapia*	CA , SA	Linares & Pérez, 1985; Peck, 1999
Digitaria decumbens Stent	AF	C_2H_2	7–34 kg ha^{-1} year^{-1}	Weier, 1980	*Aeneolamia, Prosapia*	CA, CB, SA, US	Fagan & Kuitert, 1969; Linares & Pérez, 1985; Mislevy, 2002
Hemarthria altissima (Poir) Stapf & C.E. Hubb.	AF	C_2H_2	7 kg/ha^{-1} year^{-1}	Maasdorp, 1987	*Prosapia*	US	Adjei, 1999; Mislevy, 2002
Oryza sativa L.	AS, AE	$^{15}N_2$	0–72% total N	Yoneyama *et al.*, 1997	*Aeneolamia, Callitettix, Deois, Locris, Notozulia, Poophilus*	AE, AF, CB, SA	Nilakhe, 1985; Ajayi & Oboite, 1999; Li *et al.*, 2001
Panicum maximum Jacq.	AF	$^{15}N_2$	60–120 kg ha^{-1} year^{-1}	Miranda & Boddey, 1987	*Aeneolamia, Deois, Notozulia, Prosapia*	CA, SA	Coronado & Sosa, 1966; Nilakhe *et al.*, 1985; Peck, 2001
Pennisetum americanum (L.) Leeke	AF	$^{15}N_2$	N/A	Giller *et al.*, 1986	*Locris, Poophilus*	AF	Ajayi & Oboite, 1999
Pennisetum clandestinium Hochst. Ex Chiov.	AF	N-increase	N/A	Crush, 1994	*Deois, Prosapia*	CA, SA	Lepage & Monte, 1942; Peck, 1999
Pennisetum purpureum Schumach.	AF	$^{15}N_2$	106–165 kg ha^{-1} year^{-1}	Reis *et al.*, 2001	*Aeneolamia, Mahanarva, Prosapia*	CA, SA	Coronado & Sosa, 1966; Faria *et al.*, 1998
Saccharum officinarum L.	AU	$^{15}N_2$	170–270 kg ha^{-1} year^{-1}	Urguiaga *et al.*, 1992	*Aeneolamia, Clovia, Eoscarta, Mahanarva, Peuceptyelus, Poophilus, Prosapia, Ptyelus*	AE, AS, AU, CA, CB, SA	Fewkes, 1969; Dinardo-Miranda *et al.*, 1999
Sorghum bicolor (L.) Moench	AF	C_2H_2	15 kg ha^{-1} year^{-1}	Krotzsky *et al.*, 1988	*Locris, Poophilus*	AF	Ajayi & Oboite, 1999
Zea mays L.	CA	$^{15}N_2$	to 35% total N	Garcia de S. *et al.*, 1996	*Aeneolamia, Callitettix, Deois, Locris, Prosapia*	AE, AF, SA, US	Janes, 1971; Lei *et al.*, 1992; Ajayi & Oboite, 1999

Excludes all host grasses with < 5 kg ha^{-1} year^{-1} N-fixation measured quantitatively, all hosts except *Oryza sativa* have C_4 photosynthesis.
**Aeneolamia, Deois, Mahanarva, Notozulia, Prosapia* and *Zulia* are New World Cercopidae; *Callittettix, Eoscarta* and *Locris* are Old World Cercopidae; *Clovia, Peuceptyelus, Poophilus* and *Ptyelus* are Old World Aphrophoridae.
AE, east Asia; AF, Africa; AS, south Asia; AU, Australia and New Guinea; CA, Central America and Mexico; CB, Caribbean Islands; SA, South America; US, United States of America; $^{15}N_2$, nitrogen isotope assay; C_2H_2, acetylene reductase assay; N increase, increase in soil N.

Table 3. C_4 grasses (Poaceae) with spittlebug pests but no reported N-fixation.

Host grass species	Host geo-origin	Primary spittlebug pest genera*	Spittlebug pest geo-localities	Selected spittlebug pest references
Bothriochloa pertusa (L.) A. Camus	?	*Aeneolamia*	SA	Peck, 2001
Chloris guyana Kunth	AF	*Prosapia*	NA	Mislevy, 2002
Eremochloa ophiutoides (Munro) Hack	AE	*Prosapia*	NA	Shortman *et al*., 2002
Stenotaphrum secundatum (Walt.) Kuntze	NA, CA, SA	*Prosapia*	NA	Janes, 1971
Echinochloa polystachya (H.B.K.) Hitchc.	CA, CB, SA	*Aeneolamia, Prosapia*	CA	Coronado & Sosa, 1966

**Aeneolamia* and *Prosapia* are New World Cercopidae.
AE, east Asia; AF, Africa; CA, Central America and Mexico; CB, Caribbean Islands; NA, North America; SA, South America.

Quantitative measurements of associative N-fixation present uncertainties

Despite several decades of work, much of it performed or inspired by the late Johanna Döbereiner and her associates in Brazil, there is no consensus regarding the efficacy and extent of associative N-fixation in natural and agricultural systems (James, 2000; Kahn *et al*., 2002). Associative N-fixation is intrinsically difficult to quantify. The cheapest, most widely used method, the root core acetylene reductase assay, measures nitrogenase activity, not N incorporation, and is subject to considerable uncertainty (van Berkum & Bohlool, 1980). Methods based on measurement of the incorporation of heavy nitrogen (^{15}N) are expensive, subject to high experimental error, and sometimes of questionable applicability to systems outside the laboratory (James, 2000). In addition, some of these methods require the selection of a 'non-fixing' reference plant, complicating the interpretation of results (Yoneyama *et al*., 1997; Reis *et al*., 2001; Biggs *et al*., 2002). Analyses based on measured increases in plant N independent of mineral N sources are also subject to considerable error (Barkman & Schwintzer, 1998). A promising new method based on ^{15}N incorporation into chlorophyll has not yet been applied to associative N-fixing systems (Kahn *et al*., 2002). Consequently, although it is clear that associative N-fixation occurs in many grasses (Chalk, 1991; Boddey *et al*., 1996) and is economically important in Brazilian sugarcane (Baldani *et al*., 2002), its overall magnitude and importance in natural and agricultural settings remain uncertain (James, 2000; Biggs *et al*., 2002).

Even low levels of N-fixation may be ecologically important to xylem-sucking insects

Given the uncertainties inherent in measurement of associative N-fixation, it is critical to note that associative N-fixation levels inconsequential for agricultural productivity might nonetheless be ecologically and evolutionarily important to plant-insect interactions, particularly for xylem feeding insects. All associative N-fixing grasses also utilize alternative soil sources of N. None can survive on associative N-fixation alone, with the highest measured levels of associatively fixed N falling below three quarters of the total (see *Oryza sativa*, table 2). At the low end of the associative N-fixation spectrum, or in agricultural circumstances in which productivity is not limited by N availability, associative N-fixation as such might be irrelevant to host yields, but still important to the development of associated spittlebugs. For example, doubling dilute concentrations of xylem-borne organic N will make little difference in plant growth, so long as a larger supply of NO_3^-, the most accessible nitrogen source in most agricultural systems, is readily available. But the same increase would double the food supply for a xylem-feeding insect. Consequently, relatively low levels of associative N-fixation, levels insignificant to agricultural N balances, could make plants attractive to xylem-sucking insects. These insects in turn will experience strong selection to evolve an attraction to N-fixing plants.

A general hypothesis linking C_4 photosynthesis, associative N-fixation and the status of spittlebugs as major Neotropical grass pests

These observations are consistent with the following interpretation. Associative N-fixation is a significant and widespread phenomenon among C_4 grasses, affecting the ecology and evolution of the grasses themselves and their associated herbivores. Spittlebugs are attracted to N-fixing grasses with xylem sap relatively enriched in organic N compounds. These nutritionally superior hosts in turn support faster insect growth and reproduction, creating potential for high population densities and setting the stage for economically important outbreaks.

Spittlebug outbreaks are by far most pronounced, widespread and destructive in the Americas. This reflects two factors. First, the tropical Americas, in contrast to Africa, for example, are uniquely dependent on extensive, artificially planted pastures (Boddey *et al*.,1996; Ndikumana & de Leeuw, 1996). Second, when adult Neotropical spittlebugs feed on the introduced Old World grasses comprising most tropical American pastures, they not only divert nutrients and water, they actively poison the leaves (Ferrufino & Lapointe, 1989; Valério *et al*., 1996; Peck, 1999). Consequently, spittlebug infestations may cause leaf necrosis or death across whole cane fields or pastures, resulting in serious declines in yield (cane) or stocking capacity (pasture). Old World spittlebugs, which evolved with and live on Old World grasses, generally do less damage, perhaps because they have coevolved with their hosts. Conversely, the New World grass *Z. mays* suffers serious spittlebug attack in parts of China, with yield losses of up to 50% (Lei *et al*., 1992; Li *et al*., 2001), and moderate attack in West Africa (Ajayi & Oboite, 1999), but does not suffer widespread, severe attack in its home hemisphere.

Given the link between associative N-fixation and susceptibility to spittlebug attack, it is ironic that superior N-fixing ability may itself have played an important role in the sweep of the Neotropics by Old World grasses. These

Table 4. Nitrogen-fixing C_4 forage grasses (Poaceae) with associated spittlebugs but no recorded spittlebug pests.

N-fixing host grass species	Host geo-origin	Evidence for N-fixation	Quantitative estimate of N-fixation	Selected N-fixation reference	Primary spittlebug genera*	Spittlebug geolocalities	Selected spittlebug references
Andropogon gayanus Kunth	AF	N-increase	59–83 kg ha^{-1} year^{-1}	Dart, 1986	*Locris, Poophilus, Zulia*	AF, SA	Ajayi & Oboite, 1999; Peck, 2001
Axonopus compressus. (Swartz) Beauv	NA, CA, SA	C_2H_2	19 kg ha^{-1} year^{-1}	Weier, 1980	*Aeneolamia, Zulia*	SA	Linares & Pérez, 1985
Brachiaria brizantha (Hochst. Ex A. Rich.) Stapf	AF	$^{15}N_2$	0–26% total N	Reis *et al.*, 2001	*Deois, Mahanarva, Notozulia*	SA	Melo *et al.*,1984; Peck, 2001
Paspalum notatum Flugge	CA, SA	$^{15}N_2$	14 kg ha^{-1} year^{-1}	Boddey & Victoria, 1986	*Aeneolamia, Deois, Mahanarva, Prosapia, Zulia*	CA, SA, US	Linares & Pérez, 1985; Melo *et al.*, 1984
Rhynchelytrum repens (Willd.) C.E. Hubb.	AF	C_2H_2	12–63 kg ha^{-1} year^{-1}	Maasdorp, 1987	*Aeneolamia, Prosapia*	SA, US	Fagan & Kuitert, 1969
Sorghum halepense L.	AF?	$^{15}N_2$	1–5 kg ha^{-1} year^{-1}	Morris *et al.*, 1985	*Aeneolamia, Mahanarva, Prosapia*	CA, CB, SA, US	Linares & Pérez, 1985; Peck, 2001

Excludes all host grasses with < 5 kg ha^{-2} year^{-1} N-fixation measured quantitatively.
* *Aeneolamia, Deois, Mahanarva, Notozulia, Prosapia*, and *Zulia* are New World Cercopidae; *Locris* are Old World Cercopidae; *Poophilus* are Old World Aphrophoridae.
AF, Africa; CA, Central America and Mexico; CB, Caribbean Islands; NA, North America; SA, South America; US, United States of America; $^{15}N_2$, nitrogen isotope assay; C_2H_2, acetylene reductase assay; N-increase, increase in soil N.

grasses proliferated because they are more palatable and productive than their native American counterparts (Parsons, 1970; Boddey *et al.*, 1996), perhaps in part precisely because African grasses have more effective associative N-fixation. This hypothesis is consistent with evidence that associative N-fixation by native Sahel and savanna grasses is a major source of fixed N in West Africa (Robertson & Rosswall, 1986; Abbadie *et al.*, 1992). It is certain that the recent spread of N-fixing *Brachiaria* species in the Americas has been driven by their superior productivity on nutritionally deficient soils, probably due in part to their capacity for associative N-fixation (Rao *et al.*, 1996). Interestingly, grass-feeding spittlebugs are rare in South American grasslands populated by native grasses (Pickles, 1931; Myers, 1935; Pires *et al.*, 2000a) and Brazilian spittlebugs reach much higher densities on introduced *Brachiaria* species than they do on native grasses (Pires *et al.*, 2000a). This suggests that these native grasslands are intrinsically spittlebug resistant.

Special cases that reinforce the rule

The great majority of tropical grasses have C_4 photosynthesis, and many, if not most, exhibit associative N-fixation. Perhaps, it might be argued, grass-feeding tropical spittlebugs specialize on N-fixing C_4 grasses simply for lack of alternative non-N-fixing hosts. Countering this, a variety of unique spittlebug-host relationships, far removed from terrestrial tropical environments, lend additional support to the hypothesis that spittlebugs are specifically drawn to associative N-fixing grasses.

Salt marsh spittlebugs

Two spittlebugs thrive on associative N-fixing salt marsh grasses. *Philaenarcys spartina* Hamilton is restricted to *Spartina alterniflora* Loisl. and *S. patens* (Aiton) Muhl. (Hamilton, 1982), C_4 grasses that form productive monocultures in intertidal habitats in northeastern North America. *Philaenus spumarius* (L.), a highly polyphagous spittlebug, is the most important canopy feeder on *Spartina anglica* C.E. Hubb., a similar but relatively newly established West European hybrid C_4 tidal marsh grass (Jackson *et al.*, 1985). All three of these C_4 grasses flourish at high latitudes dominated by C_3 grasses, and all exhibit associative N-fixation, estimated at 105–130 kg ha^{-1} year^{-1} for *S. alterniflora*, 6–35 kg ha^{-1} year^{-1} for *S. patens* and 4–6 kg ha^{-1} year^{-1} for *S. anglica* (Teal *et al.*, 1979; Wolfenden & Jones, 1987; Dakora & Drake, 2000). Both *P. spartina* and *P. spumarius* belong to the spittlebug family Aphrophoridae and are quite distinct from spittlebugs of the Neotropical grass-feeding Cercopidae.

Schizachyrium scoparium *spittlebugs*

Prosapia ignipectus (Fitch), the northernmost species of the family Cercopidae in the Americas, feeds on the C_4 grass *Schizachyrium scoparium* (Michx.) Nash in the northeastern US and Ontario (Hamilton, 1982). *Schizachyrium scoparium* is also an important host of two Aphrophoridae spittlebugs, *Philaenarcys killa* Hamilton (Panzer *et al.*, 1995) and *Lepyronia gibbosa* Ball (Dunn *et al.*, 2002) in the American Midwest, and *P. killa* in New England (Thompson, unpublished). *Schizachyrium scoparium* has been recorded to exhibit weak associative N-fixation (1.8 kg ha^{-1} year^{-1}; Tjepkema &

Burris, 1976). These host relationships underscore the widespread association between spittlebugs and N-fixing C_4 grasses, extending it to northern habitats for the family Cercopidae and, as in the case of the salt marsh spittlebugs cited above, encompassing species from the family Aphrophoridae. The pervasiveness of spittlebug–*S. scoparium* associations suggests that N-fixation in *S. scoparium* may be greater than the modest measured level, a suggestion consistent with *S. scoparium*'s frequent abundance on nutrient poor soils.

Miscanthus sinensis *spittlebugs*

Most C_4 grasses are adapted to warm climates, or to the warmest season in mid-latitude climates, but there are notable exceptions. In addition to the *Spartina* species noted above, many highly productive C_4 grasses of the genus *Miscanthus* occur in cool climates, stimulating speculation that the advantages of C_4 photosynthesis may not critically depend on high ambient temperatures (Long, 1999). The most important agricultural *Miscanthus* species is *M. sinensis* Anderss. and its hybrids, under development in Europe as a high biomass energy source (Clifton-Brown & Lewandowski, 2002). In Japan, where it is native, *M. sinensis* is infested by nymphs of the spittlebug *Aphrophora stictica* Matsumura (Aphrophoridae) (Komatsu, 1997; K. Morishima, personal communication). *Miscanthus sinensis* is associated with endophytic N-fixing bacteria and combines high biomass production with lack of response to N fertilizer, suggesting that it exhibits associative N-fixation (Eckert *et al.*, 2001). It represents another link between spittlebugs and an ecologically unusual C_4 grass with N-fixing potential. Tantalizingly, *M. sinensis* is the alternative host for a severe, localized cicada pest of sugarcane in Okinawa, Japan (Itô & Nagamine, 1981). Cicadas, for reasons analogous to spittlebugs, may prefer N-fixing hosts (Young, 1984) and have been reported several times as pests of sugarcane (Wilson, 1987).

Spittlebugs on Amazon riparian grasses

The C_4 grass *Paspalum fasciculatum* Willd. forms large monospecific stands on the higher levels of the floodplain in the middle reaches of the Amazon. It is the only recorded food plant of the abundant spittlebug *Deois morialis* (China & Myers), which it hosts 'in veritable clouds' (Myers, 1935). Martinelli *et al.* (1992) suggest that Amazon riparian *Paspalum* species may exhibit N-fixation. If so, this would represent another example, in a unique ecological setting, of spittlebugs associated with an N-fixing C_4 grass. Interestingly, another C_4 riparian grass, *Echinochloa polystachya* (H.B.K.) Hitchc., which generally grows just riverward from *P. fasciculatum*, is the favoured local host of the spittlebug *Mahanarva tristis* (Fabricius) (Pickles, 1938). However, evidence suggests that riparian *E. polystachya* derives its N from river water (Piedade *et al.*, 1997). *Mahanarva tristis* is a minor pest of sugarcane in Venezuela and Guyana and *E. polystachya* suffers spittlebug damage in Mexico (table 3). Other species of *Deois* and *Mahanarva* are important pests of sugarcane and pasture grasses over wide areas of South America (Fewkes, 1969; Valério *et al.*, 1996). These observations suggest that N-fixation in Amazonian riparian C_4 grasses merits closer investigation.

Spittlebugs on N-fixing bamboos

Spittlebugs have been reported on bamboos, which are highly modified C_3 grasses, only five times over the past 70 years. In four cases the associations were apparently incidental. But, Lu & Xu (1992) reported that the spittlebug *Aphrophora horizontalis* Kato (Aphrophoridae) is a serious pest of bamboo species of the genus *Phyllostachys* in one area of southern China. Two years later, Gu & Wu (1994) presented evidence for associative N-fixation in *Phyllostachys meyeri* McClure and *P. pubescens* Mazel ex H. de Lehaie, the first and only reported cases of N-fixation in bamboos. This extraordinary conjunction of observations provides additional support for the hypothesis that spittlebugs have a special attraction to N-fixing grasses, even highly modified C_3 grasses well outside the ordinary host range.

Taken together with the better studied pest associations summarized in table 2, these unusual cases lend strong support to the hypothesis that associative N-fixing grasses offer special attractions for spittlebugs.

Agricultural consequences: implications for combating spittlebug pests

Breeding and selection for spittlebug resistance

If associative N-fixation plays an important role in attracting and fostering grass-feeding spittlebug pests, any factor that lessens its magnitude or disguises its existence should help deter or mitigate spittlebug attacks. Conversely, higher levels or more conspicuous advertisements of associative N-fixation should tend to attract spittlebug pests.

There is strong evidence that xylem-sucking insects respond to changes in the concentrations of organic N compounds in the xylem stream (references in Thompson, 1994). If particular compounds attract spittlebugs to C_4 N-fixing grasses, it may be possible to breed strains in which these compounds are eliminated or minimized. This approach has produced sorghum lines resistant to hemiparasitic plants of the genus *Striga*, the witchweeds of Africa and southern Asia. Resistant lines produce less sorgolactone, a *Striga* germination stimulant excreted by host roots (C.M., 1997). *Striga* plants are direct plant kingdom analogues of root-feeding spittlebug nymphs, siphoning nutrients from host root xylem tissue (Press & Whittaker, 1993). Not surprisingly, they preferentially attack C_4 grasses with N-fixing potential, including sorghum, maize, millet, rice, sugarcane and wild grasses of the genera *Brachiaria*, *Digitaria*, *Panicum*, *Paspalum* and *Pennisetum* (Parker & Riches, 1993), an array remarkably similar to the N-fixing spittlebug hosts listed in table 2.

Alternatively, there is evidence that some xylem-borne compounds deter feeding or damage nymphs (Thompson, 1994; Brodbeck *et al.*, 1999). Consequently, it should be possible to starve spittlebugs or suppress their nymphal development by manipulating root biochemistry to favour the production of antifeedants or antibiotics. In either case, changes in xylem sap content are likely to be found among some of the spittlebug resistant strains bred or discovered in selection and screening programmes for spittlebug resistance (e.g. Ferrufino & Lapointe, 1989; Lapointe *et al.*, 1992; Miles *et al.*, 1995; Valério *et al.*, 1996, 2001; Cardona *et al.*, 1999; Shortman *et al.*, 2002). Conscious application of this perspective in breeding programmes could lead to faster progress and deeper understanding of the results.

Effects of nitrogen fertilization

Nitrogen derived from associative N-fixation, other forms of biological N-fixation, and inorganic ammonium compounds is transported in the xylem in the form of organic compounds, for the most part easily assimilable by spittlebugs. Consequently, NH_4^+ fertilization should make host plants more attractive to spittlebugs. In contrast, inorganic NO_3^- fertilization has two effects on N-fixing plants. Firstly, it ordinarily suppresses N-fixation, because plants with readily available fixed mineral nitrogen do not waste energy feeding associated N-fixing symbionts. Secondly, it may lead to substitution of NO_3^- for organic N in the xylem stream, because many plants (Andrews, 1986), including C_4 grasses (Venkataramana *et al*., 1987; Jiang *et al*., 2002), transport NO_3^- to the leaves before converting it to organic N. Therefore, all other factors being equal, NO_3^- fertilized C_4 grasses should carry lower levels of xylem-borne organic compounds and attract and support fewer spittlebugs, while NH_4^+ fertilized grasses should attract and support more. This is consistent with evidence that application of NPK fertilizer to *B. decumbens* (with the N applied as NO_3^-) retards nymphal development in the spittlebug *Deois flavopicta* Stål, a serious *B. decumbens* pest (Pires *et al*., 2000b) .

Genetic engineering of C_4 capacity in C_3 plants, and attempts to increase the effectiveness of associative N-fixation in C_4 grasses

C_4 grasses have superior yield potential in many environments. This has stimulated interest in using genetic engineering techniques to transfer portions of the C_4 photosynthetic system to C_3 grasses, particularly rice (Mann, 1999; Matsuoka *et al*., 2001; Surridge, 2002; Miyao, 2003). Surridge (2002) has described the effort to create C_4 rice as perhaps the most audacious feat of genetic engineering yet attempted, and one that could underpin a second green revolution. However, were such a transfer to increase photosynthetic capacity in rice or other crops, it also would increase those crops' potential for sharing photosynthetic carbon compounds with microbial partners and stimulating associative N-fixation (Rengel, 2002). This could enhance host vulnerability to spittlebug pests, just as host vulnerability might increase from the introduction of N-fixation into non-fixing plants (Thompson, 1994).

Likewise, many workers have suggested that enhanced associative N-fixation could make major contributions to lessening dependence on chemical fertilizers (Döbereiner, 1992; Reis *et al*., 2001; Baldani *et al*., 2002), and one (Triplett, 1996) has described nitrogen-fixing maize as the 'holy grail' of N-fixation research. This has stimulated efforts to improve associative N-fixation in sugarcane (Oliveira *et al*., 2002) and maize (Garcia de Salamone *et al*., 1996; Chelius & Triplett, 2000; Riggs *et al*., 2001; Estrada *et al*., 2002), among other crops, through artificial inoculation with N-fixing bacteria. If these enhancement efforts succeed, they too might produce crops that are more vulnerable to spittlebugs, unless there are coordinated efforts to engineer other characteristics that deter xylem feeding pests.

Grass-feeding spittlebugs and some general hypotheses regarding C_4 herbivores

The proliferation of spittlebugs on C_4 grasses offers a clear counter example to the hypothesis (Caswell *et al*., 1973) that C_4 plants are less nutritious and attractive to herbivores than their C_3 counterparts. While studies with other herbivores have produced variable and inconclusive results (Heckathorn *et al*., 1999; Scheirs *et al*., 2001), spittlebugs decisively favour C_4 grasses over C_3 grasses in natural and agricultural settings. The only notable exception to this pattern occurs in Europe, where grass-feeding spittlebugs of the genus *Neophilaenus* (Aphrophoridae) and nymphs of *Haematoloma dorsatum* (Ahrens) and *Cercopis vulnerata* Rossi (both Cercopidae) live primarily or exclusively on C_3 grasses. Europe, however, escaped the transition to C_4 enriched floras that swept the rest of the world at the end of the Miocene (Cerling *et al*., 1998, and see below) with the result that most European grasses have C_3 photosynthesis (Scheirs *et al*., 2001). European spittlebugs have had little evolutionary opportunity to evolve preferences for C_4 grasses, and they have not done so.

On the other hand, spittlebug feeding patterns are consistent with two related hypotheses: that C_4 plants are more susceptible to generalist herbivores, and that C_4 plants favour herbivores with larger body size (Heckathorn *et al*., 1999). Spittlebugs, on average, have broader host ranges (Thompson, 1994) and larger body sizes (Novotny & Wilson, 1997) than their phloem-sucking counterparts among related insect groups.

C_4 photosynthesis, atmospheric carbon dioxide levels, and the evolution of spittlebug pests

C_4 plants play a major role in grassland and savanna floras covering about one third of the earth's land areas. This is a very recent geological development. C_4 grasses have been abundant for only the last 6–8 million years (the oldest date back only 12–13 million years), apparently because only then did atmospheric carbon dioxide fall to levels that favoured the evolution and spread of grasses with C_4 photosynthesis. C_4 dicots may be even newer, evolving during the Pleistocene in response to extremely low carbon dioxide levels during relatively short glacial maxima (Cerling, 1999).

The New World grass-feeding Cercopidae are species rich and taxonomically difficult. Despite their diversity, they show relatively modest morphological differentiation, making it hard to separate genera and species (G.S. Carvalho & M.D. Webb, unpublished). This is consistent with the hypothesis that they underwent a rapid evolutionary radiation in recent geological time, probably in direct response to the proliferation of C_4 grasses. In contrast, spittlebugs are rarely reported on C_4 dicots, perhaps because C_4 dicots evolved so recently, or perhaps because they have not evolved associative N-fixation (there are no reported cases).

The widespread grasslands and savannas of the biologically novel 'C_4-world' (Cerling, 1999) promoted human as well as spittlebug evolution, and the subsequent development of agriculture led to the domestication and/or wide dissemination of many C_4 grasses. Humans have replaced vast areas of tropical and subtropical C_3 forest and bush with pastures and fields dominated by C_4 grasses. This presents continuing ecological and evolutionary opportunities for grass-feeding spittlebugs, including potential for the development of new spittlebug pests. But there is a counter trend that may prove to be significant. Atmospheric carbon dioxide levels are now about 350 ppmV, up from about 280 ppmV at the beginning of the industrial revolution. Taken as an isolated factor, increasing carbon dioxide concentrations should favour C_3 over C_4 plants. However, global warming promoted by rising carbon dioxide levels could favour the spread of warmer habitats in which C_4 plants have an intrinsic advantage, or shift winter temperatures to a greater degree than summer temperatures, with unpredictable net results, for both C_3 and C_4 grasses and the spittlebugs they host (Collatz *et al.*, 1998; Brooks & Whittaker, 1999; Fielding *et al.*, 1999; Sage, 1999).

Nevertheless, all evidence suggests that spittlebugs will continue to be major pests of tropical grasses and sugarcane for the foreseeable future and that understanding crop–insect interactions in the context of N nutrition will provide important insights into spittlebug proliferation and control.

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